DOCTORAL DISSERTATION

The mechanism, characteristics and efficiency of a new vertical flow labyrinth (VFL) device for treating various wastewaters

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Preface

This study constructed a vertical flow labyrinth (VFL) wastewater treatment method based on multi-source data. Taking Beijing and Huzhou, China, as examples, the performance and mechanism of multi-source wastewater treatment were explored from three levels: anaerobic, anoxic, and aerobic. The superiority of the VFL device is explained from the physicochemical and biological perspectives. From two sewage sources and two comparative experiments, the research proposes a corresponding strategy for environmental protection based on VFL to provide a reliable theoretical reference for the current development of advanced sewage treatment.

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Research Achievements during PhD

1. Xiaoxuan Wang, Weijun Gao*. Research on small sewage treatment technology. Rome conference
2. Xiaoxuan Wang, Jinming Jiang, Weijun Gao*. Reviewing textile wastewater produced by industries: Characteristics, environmental impacts, and treatment strategies. Water Science and Technology. 2022. Accept.
3. Xiaoxuan Wang, Xindong Wei, Weijun Gao, Jinming Jiang. Study on treatment of industrial wastewater with an anaerobic reactor. FEB. 2019.

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ABSTRACT

Due to high ammonia nitrogen and complex water quality characteristics, landfill leachate has caused significant difficulties in leachate's biological treatment. Therefore, it is essential to develop an efficient and energy-saving leachate natural treatment process. In addition, printing and dyeing wastewater is one of the leading industrial wastewaters with large output, strong alkalinity, high chroma, high organic concentration, complex components, and poor biodegradability. The textile industry is one of the most intensive industries in chemical products whose wastewater contains hazardous dyes, pigments, dissolved/suspended solids, and heavy metals. Hence, it is essential to effectively treat the wastewater generated by this industry before releasing it into the environment. Though textile wastewater treatment has made tremendous progress, the advanced treatment methods should be improved further to make them economically viable and friendly. Based on this, starting from landfill leachate and sand washing wastewater, this paper uses a new vertical flow labyrinth (VFL) device to investigate the effect of seasonal temperature on water quality degradation performance. On this basis, the superiority of the VFL unit and the anaerobic/anoxic/aerobic (AAO) process in the treatment of sand-washing wastewater was compared.

Using the VFL treatment process for high-concentration landfill leachate with high efficiency is economical and reasonable. The VFL device can effectively remove the high COD concentration, and the removal rate can reach 86.5%. The effluent COD was stable in the whole stage, and the concentration fluctuation of the influent did not affect the effluent COD concentration. The average concentration of NH_4^+ in the effluent is 15.5 mg/L, which meets the requirements of China's national secondary emission standard, where the NH_4^+ concentration is lower than 25 mg/L, and the NH_4^+ removal rate is as high as 99.3%. The degradation effect of total phosphorus is obvious. The total phosphorus concentration in each stage was relatively stable. The conductivity of the influent water is significantly higher than in other states. The VFL device degrades volatile fatty acids. During the treatment of landfill leachate by VFL, the environmental conditions in the anoxic section can provide a more suitable attachment site for microorganisms, accelerate the metabolism of microorganisms, and thus promote the increase of MLVSS in the sludge mixture, and the growth rate is the fastest. The activated sludge in the anaerobic, anoxic, and aerobic stages all have irregular shapes, irregular edges, and brown color, and the color depth depends on the density of the sludge. Moreover, the activated sludge is flocculent, the internal density of the sludge is uneven, the center density is large, the edges are sparse, and there are still many flocs around the sludge that gather

towards the center of the sludge.

Metagenomic sequencing technology was used to analyze the microbial community structure of sludge samples in the anaerobic, anoxic, and aerobic sections of the long-running VFL unit to treat landfill leachate. As the seasonal temperature decreases, it is not conducive to the reproduction of microorganisms involved in the degradation of landfill leachate, which leads to a relative reduction in the microbial diversity in the VFL unit. At the phylum level, Proteobacteria, Actinobacteria, unclassified_Bacteria, Deinococcus-Thermus, Chloroflexi, Ignavibacteriae, Planctomycete, Bacteroidetes were the dominant bacteria. At the genus level, *Thauera*, *Ignavibacterium*, *Nitrosomonas*, *Truepera*, *Pseudofulvimonas*, *Lewinella* were the dominant communities.

After VFL treatment, BOD_5 in sand washing wastewater was effectively degraded, and the average degradation rate of BOD_5 was as high as 86.3%. BOD_5 tends to be stable in each sampling section of VFL, and the change range is not extensive. The NH_4^+ concentration at each stage was relatively stable during the sampling period. The average removal rate of total nitrogen in the anaerobic stage was 44.3%, the average removal rate of total nitrogen in the anoxic stage was 66.8%, the average removal rate of total nitrogen in the aerobic stage was 77.8%, and the average removal rate of total nitrogen in the effluent was 80%. Overall, the total nitrogen removal rate was better during the long-term stable operation. The total phosphorus degradation rate in the effluent reached 66.7%. The pH in the VFL device did not change much and was relatively stable. Comparatively, the BOD_5 of sand washing wastewater has a small fluctuation range in the effluent stage, aerobic stage, and anoxic stage of the AAO process; however, in the influent and anaerobic phase, the BOD_5 fluctuation range is more extensive. When the AAO process treats sand washing wastewater for a long time, BOD_5 to COD in the inlet section is always greater than 0.3, which indicates that biological methods can effectively treat the sand washing wastewater. Compared with the concentration of NH_4^+-N in the influent, the NH_4^+-N in the effluent was effectively degraded, the degradation rate reached 88%, and the degradation effect was better. NO_3^--N effluent concentration is higher than influent concentration. NO_3^--N changes very smoothly in the influent, anaerobic, and anoxic sections. On the contrary, NO_3^--N fluctuates significantly in the aerobic and effluent sections. The degradation rate of phosphorus in the effluent section reached 66.7%. In general, in terms of the performance of pollutions removal and economic benefits, the VFL device was better than AAO process.

Besides, the Simpson index of each stage of the VFL device is smaller than that of the AAO process. Proteobacteria was the main dominant at the phylum level, which was the most in the aerobic section of the VFL device, reaching 77.76%. The microbial communities of the VFL plant and the AAO process were different at the family level. Sphingomonadaceae were detected in the highest number in the AAO process. Anaerolineaceae (phylum Chloroflexi) predominate in VFL installations. *Novosphingobium* has a significant advantage in the AAO process. *unclassified_Rhodocyclaceae* had a larger proportion in VFL. In addition, anammox functional genes, genes involved in nitrification, denitrification, and nitrogen fixation were detected.

Moreover, green building can effectively integrate resources and environmental issues, so as to solve the problem of harmonious development of human and environment to the greatest extent. At present, green buildings mainly recycle water resources through reclaimed water reuse and rainwater reuse. The VFL technology and the AAO technology have high pollutant removal rates and high environmental benefits in the operation of sand washing wastewater treatment, so VFL is a good choice for green building sewage treatment technologies. The engineering construction and operation phases of the VFL treatment technology of the selected treatment plant were analyzed based on the whole LC theory. The results show that the engineering cost of the treatment technology is 54,699,000 yuan, and the average annual engineering and construction investment is 1,727,000 yuan, which is about 0.34 yuan/m^3 of wastewater. The calculation of operation costs takes into account energy consumption, chemical consumption, sludge disposal, major maintenance and personnel costs, combined with the actual consumption during technology operation, the annual operation fee is 19.3 million yuan, and the unit operation fee is 3.8 yuan/m^3. The revenue of this project comes mainly from wastewater treatment and supplying reuse water to enterprises in the park, with a total annual profit of about 23.72 million yuan. In addition, the average annual revenue is calculated to be 2,693,000 yuan based on the combined annual project investment, annual operation expenses and total profit. The above results show that a series of VFL-based wastewater treatment technologies are profitable as green buildings. More importantly, the profits will be even greater when the environmental benefits of treated and reused wastewater are additionally taken into account. VFL can be used as a treatment technology for green building sewage reuse. The effluent quality of VFL meets the reuse standard and is mainly used for greening and irrigation. The VFL technology is evaluated in five aspects: technology selection evaluation, location evaluation, material selection evaluation, engineering construction evaluation and construction cost evaluation, and is hereby recommended for use as the sewage treatment technology in green buildings.

Keywords: Landfill leachate; Printing and dyeing wastewater; Removal mechanisms; Vertical flow labyrinth (VFL); Biodiversity

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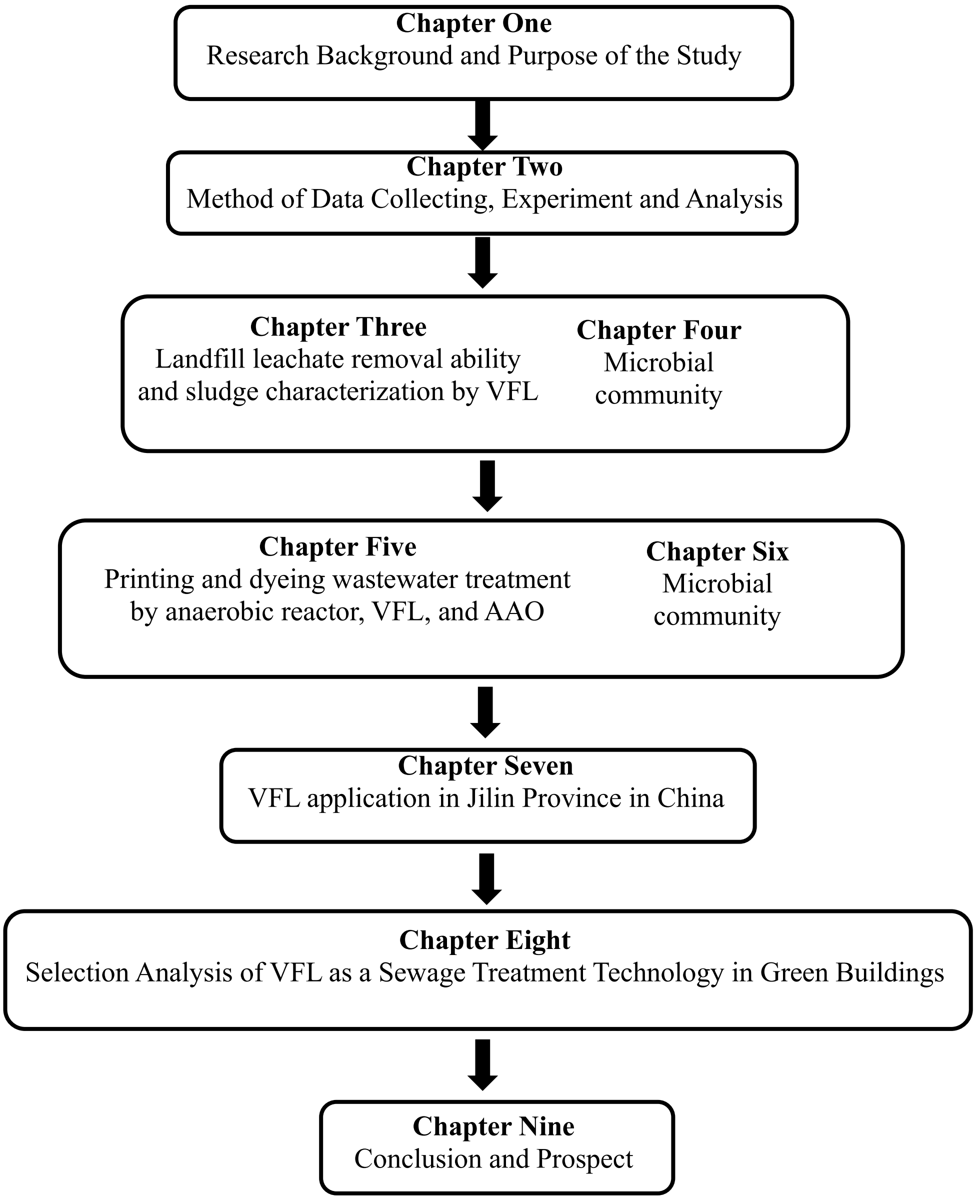

NOMENCLATURE

VFL Vertical flow labyrinth

COD Chemical oxygen demand

SS Suspended solids

BOD Biochemical oxygen demand

TDS Total dissolved solids (TDS)

C=C Double bonds

MF Microfiltration

NF Nanofiltration

RO Reverse osmosis

AOPs Advanced oxidation processes

$SO_4^{\cdot -}$ Sulfate radicals

ClO^- Hypochlorite

O^3 Ozone

EF Electro–Fenton

RM Redox mediators

PDDA Polydiallyldimethyl ammonium chloride

MBRs Membrane bioreactors

SRT Solid residence times

AAO Anaerobic-Anoxic-Oxic

NO_3^- Nitrate nitrogen

NO_2^- Nitrite nitrogen

OTUs Observed species index

UASB Up-flow anaerobic sludge bed

EGSB Expanded granular sludge bed

BOD_5 Five-day biochemical oxygen demand

AOB Ammonia oxidizing bacteria

TP Total phosphorus

VSS Volatile suspended solid

DOC Dissolved organic carbon

AOX Absorbable organic halogen

DS Dissolved solids (DS)

TiO_2 titanium dioxide (TiO_2)

UF Ultrafiltration

CA Cellulose acetate

CF Coagulation/flocculation

HO· Hydroxyl radicals

MnO_4^- Permanganate

ClO_2 Chlorine dioxide

ZVAl Zero-valent aluminum

NaClO Sodium hypochlorite

PAC Polyaluminum chloride

MFCs Microbial fuel cells

FS Flat plate

AZR Azoreductase

MLSS Mixed liquor suspended solids

MLVSS Mixed liquid volatile suspended solids

NH_4^+-N Ammonia nitrogen

POME Palm oil mill effluent

IC Internal circulation

HRT Hydraulic retention time

AAO Anaerobic/Anaerobic/Aerobic

HF Hollow fiber

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Chapter 1

RESEARCH BACKGROUND AND PURPOSE OF THE STUDY

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1.1 Research Background and Objects

1.1.1 Generation and hazards of printing and dyeing wastewater

Various scientific fields have made immense progress in the 21st century. Extensive research has been carried out on diverse topics in Environmental Science and Engineering research in the last 20 years. Large amounts of wastewater are generated daily from the textile, cosmetics, paper, rubber, leather, and printing industries [1]. It is difficult to treat the toxic and complex textile wastewater produced by industries. Currently, water contamination caused by the wastewater released from the textile industry poses a threat to economic growth. As highly water-soluble and toxic substances (microbial pathogens and organic dyes) are present in the discharged water, the wastewater directly contaminates the natural ecosystem and reduces the availability of clean and fresh water that can be utilized for drinking. The complex and stable structure of the dyes makes the degradation of dyes (present in wastewater and other complex substrates) difficult. The mineralization of dyes, presence of organic compounds, and toxicity of the wastewater released from textile and dye manufacturing industries negatively affect the environment. Therefore, it is essential to gain practical knowledge and develop methods to effectively treat textile wastewater to save the environment [2].

The printing and dyeing industry is one of the most polluting industries. Printing and dyeing wastewater is produced in all aspects of the production and processing of the textile dyeing and finishing industry. The composition of pollutants is different, among which the dyeing wastewater pollution is more serious [3]. Printing and dyeing wastewater comprises desizing wastewater, smelting wastewater, bleaching wastewater, mercerizing wastewater, dyeing wastewater, printing wastewater, soaping wastewater, and finishing wastewater (Table 1-1) and pulping, scouring, bleaching, mercerizing and other processes), dyeing process, printing process, and finishing process. Most of the pollutants in printing and dyeing wastewater are organic compounds, which vary with the type of fiber used and the processing technology. In general, the pH value of printing and dyeing wastewater is 6-10, chemical oxygen demand (COD) is 400-1000 mg/L, biochemical oxygen demand (BOD) is 100-400 mg/L, suspended solids (SS) is 100-200 mg/L, and chromaticity is 100-400 times [4].

(1) Pre-treatment wastewater

Desizing, scouring, bleaching, and mercerizing are the main processes in pre-processing cotton fabrics. Desizing wastewater contains high organic matter content; COD can reach more than 1200 mg/L, or even several thousand, mainly contains slurry, amylase, etc., but its biochemical properties are not very poor, and it is relatively easy to handle, but when artificially synthesizing slurry, Poor biodegradability, difficult to operate, and a small amount of wastewater; high turbidity, many suspended solids are insoluble in water [5]. The brewing wastewater is mainly a large amount of water. The brewing process will use a lot of water, and the temperature of the brewing water is high, and the temperature of the wastewater produced is also high. The process will add surfactants, slurries and natural ingredients, resulting in COD and BOD. high. Bleaching wastewater has chemical oxidants, a large amount of water, and good biodegradability. Mercerizing wastewater has a small amount of water but is highly alkaline and contains sodium hydroxide chemicals.

(2) Dyeing wastewater

In dyeing fabrics by adding chemical agents such as surfactants, dyes, hydrosulfites, anhydrous powder, etc., the dyes that are not combined with the fabrics, detergents, and auxiliaries enter the wastewater to form dye wastewater. This type of wastewater has the characteristics of solid alkalinity, high chroma, large water volume, high pollutant concentration, and refractory degradation. Its COD value is about 300-700 mg/L, B/C is less than 0.2, and its biodegradability is poor.

(3) Printing wastewater

Only a tiny part of a large amount of slurry in the printing process is combined with the fabric, and the rest of the slurry goes into the wastewater. Therefore, the printing wastewater contains auxiliaries, dyes, and slurry with high concentrations of pollutants, COD, and BOD. It has high water content, a large amount of water, and complex composition.

(4) Finishing wastewater

The finishing process refers to making the fabric soft, fireproof, and waterproof by using chemical reagents such as resin, formaldehyde, softener, etc. This type of wastewater generally contains wax, lint, pulp, oil, etc., and the amount of water is small. The quality and quantity of mixed sewage produced by the entire printing and dyeing enterprise have little impact.

Table 1-1. Wastewater and components are produced in each process of a typical printing and dyeing process.

Process	Addition	Wastewater and composition
Desizing	Amylase or sulfuric acid	Desizing wastewater: dilute slurry, slurry decomposition, such as glucose
Scouring	Sodium hydroxide, detergent	Cooking wastewater: surfactant, oil, wax
Bleach	Hydrogen peroxide, chlorine, hypochlorite, alkali	Bleaching wastewater: pigment, surfactant, salt
Silk	Sodium hydroxide	Silk wastewater
Staining	Dye, surfactant, anhydrous and other chemical reagents	Dyeing wastewater: waste dyes, surfactants, chemicals
Finishing	Softeners, starch, resin, formaldehyde, and other chemicals	Finishing wastewater: water chemicals, surfactants

Substandard discharge of dye wastewater will deteriorate aquatic ecosystems and endanger human life, health, and safety [6]. The dye enters the natural environment, reducing the transparency of the water body, reducing the penetration of light, and ultimately destroying the balance and stability of the entire aquatic ecosystem. Some dyes themselves and their incomplete degradation products are teratogenic, carcinogenic, and mutagenic. Some chemical substances in dye wastewater are volatile to a certain extent. If people accidentally inhale it around it, it will cause different degrees of damage to the human respiratory system. Mild cases may cause eye burns, and severe cases may even cause permanent eye damage and blindness [7]. After drinking, printing, and dyeing wastewater by mistake, it will cause damage to the human digestive system, central nervous system, and liver. If the human body uses it for a long time, the consequences are extremely serious. Among them, azo fuels can enter the human body's blood through physical contact or breathing, inhibit the formation of hemoglobin, and then lead to abnormal hematopoietic function, decrease or even disappearance of blood regeneration ability.

1.1.2 Characteristics of dyeing wastewater

It is essential to characterize textile wastewater to develop effective treatment methods and process flow. Various raw materials, such as cotton, synthetic fibers, and wool, are used in the textile industry. Wastewater is primarily produced during four steps: pretreatment, dyeing, printing, and functional finishing (Fig. 2 presents the possible contaminants and the nature of effluent discharged at each step of the industrial process). The percentage of a definite parameter for characterization of textile wastewater is included COD, pH, color, suspended solids, BOD_5, N-NH_x, total phosphorus (TP), TKN, conductivity, metals, total oxygen demand (TOC), Cl^-, total dissolved solids (TDS), grease, alkalinity, surfactants, hardness, volatile suspended solid (VSS), sulfide, N-NO_x, total solids, turbidity, dissolved organic carbon (DOC), absorbable organic halogen (AOX), TC, Org. N [8]. Composite textile wastewater is primarily characterized by analyzing BOD, COD, SS, and dissolved solids (DS) [9]. The classic characteristics of conventional textile industry wastewater are presented in Table 1-2. Data analysis reveals that the COD value corresponding to mixed wastewater is significantly high.

Table 1-2. Characteristics of conventional textile industry wastewater [9, 10].

Code	Parameters	Values
1	pH	6.0–10.0
2	Temperature (℃)	35–45
3	Biochemical oxygen demand (mg/L)	80–6000
4	Chemical oxygen demand (mg/L)	150–12000
5	Oil and grease (mg/L)	10–30
6	Total suspended solids (mg/L)	15–8000
7	Free ammonia	< 10
8	Total dissolved solids (mg/L)	2900–3100
9	Chloride (mg/L)	1000–1600
10	Sodium (mg/L)	70%
11	Trace elements (mg/L)	< 10
12	Silica (mg/L)	< 15

13	Total Kjeldahl Nitrogen (mg/L)	70–80
14	Color (Pt-Co)	50–2500

Notably, the significant contaminants in textile wastewater are produced during dyeing and finishing processes. Aromatic hydrocarbons and heterocyclic dyes are commonly used in the textile industry [2]. A dye molecule consists of two parts: the dye group and the dye auxiliary pigment [11]. When the dye molecules are exposed to light, the structure containing double bonds (C=C) oscillates to absorb light and produce visible colors [12]. Dye at low concentrations can also exhibit highly intense color [11, 13]. The complex and stable structures exist in textile wastewater and any kind of complex substances. Dyes can be classified into various categories based on their characteristics. They are primarily classified as ionic and non-ionic dyes [14]. Ionic dyes are direct, reactive, and acidic dyes. Non-ionic dyes remain dispersed as they do not ionize in a water-borne medium [15]. Methyl orange, acid red-B, rhodamine-B, Prussian red, alizarin red, Congo red, orange green, rose Bengal, and basic yellow 28 are ionic dyes [16]. Textile dyes are acidic, alkaline, direct, dispersed, active, sulfur, or reducing dyes (Table 1-3) [12]. Mordant dyes are negatively charged, and alkaline dyes are positively charged. The dyes are active if anionic dyes are used in the textile industry, medium if metal ions are present, reduced if derived from natural indigo, and disperse if non-ionic [17]. Direct dyes are the most popular class of dyes, as they are easy to use, exhibit a wide range of colors, and economically friendly. The structures of most direct dyes contain di-azo and tri-azo moieties. The maximum capacity of colors can be observed for the azo dye (percentage of dyes belonging to this class: 60–70%) [15].

Table 1-3. Dye classification and methods of application [12, 18, 19].

Dye class	Characteristics	Substrate (fiber)	Metals in dyes	Dye-fiber attachment mechanism	Method of application	Dyeing method
Acid	Anionic, water-soluble	Nylon, wool, silk	Copper, lead, zinc, chromium, cobalt	Ionic bond, Van der Waals	Suitable for neutral to acidic dye baths.	Fiber is placed in an acidified aqueous medium (pH: 2.5–7; dye temperature: 100–110 ℃).
Basic	Cationic, water-soluble	Acrylic, nylon, silk cotton, wool	Copper, zinc, lead, chromium	Ionic bond	Suitable for acidic dyebaths.	Fiber is placed in an acidified aqueous dye bath (pH: 5–6.5; temperature 105 ℃).
Direct	Anionic, water-soluble	Cotton	Copper, lead, zinc, chromium	H-bond	Application from neutral or micro-alkaline baths containing additional electrolytes.	Fibers are placed in a slightly alkaline medium; the electrolyte is used at 100 ℃.
Disperse	Colloidal dispersion, very low water solubility	Polyester, nylon, acetate, cellulose, acrylic	None	Solid solution formation	Fine water dispersions are often applied by high temperature–pressure or using lower temperature carrier methods.	Fiber is placed in an acidified dye bath (pH: 5.5; temperature: 130–210 ℃).
Sulfur	Colloidal, insoluble	cotton	-	Dye precipitated fiber	Aromatic substrate adds sodium sulfide and re-oxidizes the fibers into insoluble sulfur-containing products.	Fiber is placed in a bath; dye is dissolved in alkaline sulfur, which is replaced by the electrolyte and deposited in air or peroxide central site.
Reactive	Anionic, water-soluble	Cotton, silk, nylon, wool	Copper, chromium, lead	Covalent bond	Reactive sites present on the dyes react with the functional groups present in the fiber and bind the dye covalent bonds under the influence of heat and pH (alkaline).	Fiber placed in dye bath; salt added to displace dye; alkali added to induce reaction between dye and fiber.
Vat	As sulfur dye	Cotton	None	As sulfur dye	Water-insoluble dyes are dissolved by reducing them using sodium hydrosulfite, then exhausted on fiber and re-oxidized.	As sulfur dye.

In addition to harmful dyes, wastewater produced by the textile industry also contains various pigments, heavy metals, sulfates, oils, surfactants, and chlorides [20] (Fig. 1-1). These contaminants can adversely affect aquatic life and water quality. Heavy metals have often been used during the process of dye fixation and also in dyes. It has been reported that the metal units present in dyes help impart color so that the dues can be used as textile colorants. Textile wastewater contains trace amounts of metals such as Cu, Cr, As, and Zn, which harm the environment [10, 21]. These metals can bind with organic dyes and fibers [22]. The primary contaminants in textile wastewater are high suspended substances, COD, acidity, heat, color, and other soluble substances [9]. In general, textile wastewater exhibits intense color and is characterized by high BOD/COD values and high saline loading [2]. The BOD/COD ratio for composite textile wastewater is approximately 0.25. This indicates that wastewater contains a large amount of non-biodegradable organic substances. [9]. As reported by Paździor et al. [23], industrial textile wastewater treatment approaches are studied using various effluents generated during the execution of different processes within the dye-house. These effluents are collected under conditions of equilibrium or in neutralization pools. The pollution at a lower concentration may be present in the effluents.

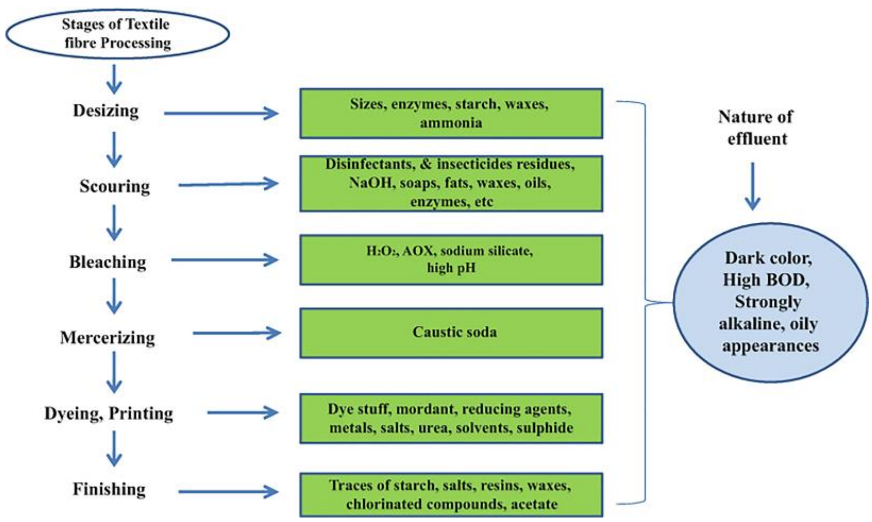

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Figure 1-1. Possible pollutants present and the nature of effluents discharged at each step of the industrial process; reprinted with permission from Ref. [10].

In general, printing and dyeing wastewater have the characteristics of large water volume, high content of organic pollutants, high chemical toxicity, and deep chromaticity. The orders and seasonal production of enterprises also make the water quality components complex and change significantly, especially in printing and dyeing wastewater. The dyes, auxiliaries, and reagents will seriously pollute the environment. Printing and dyeing wastewater contains trace organic compounds with significant and persistent chronic ecotoxicity to the environment, such as aniline, nonylphenol, perfluorooctanoic acid, polychlorinated phenol, chlorinated benzene, phenol, phthalate plasticizer, polycyclic aromatic hydrocarbons, etc. Various printing and dyeing auxiliaries have resulted in many environmental problems [24]. These dyeing wastewaters will be directly or indirectly discharged into natural water bodies, and the residual dyes in the water will absorb a large amount of light and light sources mapped to the water surface, absorbing and consuming oxygen-containing substances in the water body. In addition, printing and dyeing dyes usually generate aromatic ammonia compounds, nitro compounds and halides due to amino, nitro, halogen and other substances in aromatic compounds, which have high biological toxicity and cause damage to the local ecological environment [25]. Therefore, COD, TN, NH_3-N and chromaticity are common effluent control indicators for printing and dyeing wastewater. With the continuous advancement of printing and dyeing technology, more and more various dyes, auxiliaries, and chemicals have been used, resulting in more complex components of pollutants in wastewater than before, resulting in more and more harmful, toxic, and difficult to degrade components in sewage, which increases the difficulty of treatment of printing and dyeing wastewater and causes more harm to the ecological environment.

1.1.3 Environmental impact and toxicity of textile wastewater

It has been established that wastewater significantly pollutes the environment [26]. Wastewater can pollute the surface water, groundwater, soil, and air. Numerous textile and dyeing factories are found in developing countries, where wastewater is often poorly treated [22]. Textile wastewater is hazardous to the environment as it contains carcinogenic, toxic, mutagenic, and difficult-to-degrade compounds [27]. It has been reported that approximately 2000 different types of chemicals (dye, transfer agents, etc.) find their use in the textile industry [22]. Dyes are one of the primary contaminants present in wastewater released by the textile industry [28]. Since the first synthetic dye discovery in 1856, more than 10000 different textile dyes (estimated annual output: 8×10^5 metric tons) have been commercialized worldwide. Approximately half of these dyes fall under the category [15]. Many of these toxic dyes eventually enter the waterways, causing severe environmental problems [29]. It has been reported that the textile industry utilizes large amounts of water during textile processing. The percentage of unsafe dyes present in the wastewater system ranges from 5–10% [30]. An estimated 280,000 tons of textile dyes are discharged (annually) worldwide through industrial wastewater [31]. Approximately 10 to 15% of the dyes are released into the environment during various substrates staining. The substrates include synthetic fibers, natural textile fibers, plastics, leather, paper, mineral oils, waxes, specific types of food items, and cosmetics [32]. It is tough to handle textile wastewater as it is characterized by high content variability and color strength. The color of these dyes can potentially change the extent of turbidity causes, COD value, pH value, and temperature of the water body [18]. It is estimated that approximately 2% of the dyes are directly released into the aqueous effluent and 10% of the dyes are therefore lost during the process of coloring [10].

The maximum amount of harm to the environment is caused when sunlight is absorbed and reflected by the water system containing dyes. Light absorption changes the algal photosynthetic activity, altering the food chain [10]. The discharge of these harmful substances into the soil environment and aquatic system results in low light transmittance and low oxygen consumption. This can negatively influence the process of photosynthesis and marine life [2]. Apart from adverse aesthetic effects, these dyes can harm organisms by exerting carcinogenic and mutagenic effects [33]. It was estimated that out of 3200 azo dyes used, 130 dyes could be used to produce carcinogenic aromatic amines following the processes of reduction and degradation [32]. Contact with azo dyes can result in skin, lung, and gastrointestinal problems. These dyes can enter the body through the digestive system and destroy the roots of hemoglobin and DNA substances. The substances can induce cancer in humans and animals [16]. Contact to leukemia with multiple colors affecting the circulatory, respiratory disease, allergic reactions, neurobehavioral, and immune suppression disorders. Carcinoma of the kidneys, liver, and urinary bladder has been reported in textile workers [16]. Results from experimental studies conducted on animal models by Raj et al. [34] indicate that the main category of textile dyes, i.e., azo dyes, are directly associated with human bladder cancer, splenetic sarcomas, and hepatocarcinoma (the primary cause of chromosome aberration in mammalian cells).

Mathur et al. [35] assessed the mutation-causing ability of textile dyes from Pali (Rajasthan) by conducting an Ames bioassay. In their study, 7 days were completed for their mutagenicity by

Ames assay, using strain TA 100 of *Salmonella typhimurium* [35]. The results indicated that only 1 dye, Violet, exhibited no mutational activity. The use of the remaining 6 dyes resulted in mutation [35]. It has also been reported that bioassays are sensitive and reliable methods that can be conducted to determine the toxicity of industrial wastewater. Hence, they can assess the efficiency of emerging tools [36]. The relative sensitivity of biological assays toward textile wastewater is arranged in descending order: plant enzymes > bacteria > algae ≈ daphnids ≈ plant biomass ≈ germination rate > fish. Significant effects on genetic toxicity were not observed [36]. The aquatic toxicity of a series of unique direct dyes containing benzidine congeners, 2,2'-dimethyl-5,5'-dipropoxybenzidine, and 5,5'-dipropoxybenzidine, and the toxicity of a commercial dye (C.I. Direct Blue 218) were assessed by conducting acute toxicity studies in the presence of *Daphnia magna* [32]. The results revealed that C.I. Direct Blue 218 was highly toxic to daphnids. It was more harmful than the unmetallized direct dyes. In addition, the results also showed that the assay conducted with *D. magna* could be effectively used to assess the aquatic toxicity of dyes [32]. Villegas-Navarro et al. [37] used the crustacean *Daphnia magna* as a sensor organism and LC_{50} as the standard for measuring the toxicity of textile effluents (treated and non-treated). The results indicated that all the five textile industries could produce toxic non-treated water (ATU: 2.1–25.4). The treated water was also toxic (ATU: 1.5–7.2). This suggested that the treatment plants and methods used by the five textile industries to remove toxic water were not highly efficient [37]. Sharma et al. [38] used Swiss Albino rats to assess the toxicity of the wastewater generated from the textile industry. Table 1-4 presents information on the toxicity of some commonly used azo dyes.

Table 1-4. Most commonly used azo dyes and the health hazards caused by them [39, 40].

No.	Dyes	Toxicity and side effects
1.	Acid Fuchsin	Acute oral toxicity and neurotoxicity
2.	Alizarin	Clastogenicity, hypersensitivity, environmental toxicity, estrogenicity, genotoxicity, photoinduced toxicity, mutagenicity, and acute oral toxicity
3.	Auramine O	DNA damage, mutagenicity, cytotoxicity, carcinogenicity, and genotoxicity
4.	Congo Red	Toxic toward bacteria, yeast, algae, protozoa. Causes genotoxicity, microbial toxicity, carcinogenicity, cytotoxicity, and mutagenicity
5.	Crystal Violet	Chromosome damage, mutagenicity, genotoxicity, percutaneous toxicity, and acute oral

		toxicity
6.	Orange-II	Carcinogenicity, fish toxicity, mutagenicity, and other environmental toxicity
7.	Eosin Y	Environmental toxicity, carcinogenicity, mutagenicity, cardiotoxicity, nucleic acid damage, microbial toxicity, pulmonary toxicity, skin toxicity, and reproductive toxicity
8.	Methyl Orange	Mutagenicity, carcinogenicity, and genotoxicity
9.	Malachite Green	Genotoxicity, mitochondrial toxicity, chronic toxicity, DNA damage
10.	Methylene Blue	Microbial toxicity, mutagenicity, hematotoxicity, nucleic acid damage, teratogenicity, photodynamic toxicity, reproductive toxicity, and effluent toxicity
11.	Rhodamine 6G	Carcinogenicity, mutagenicity, genotoxicity, and DNA damage

The discharge of untreated or semi-treated colored sewage into the nearby water affects the extent of penetration of light and oxygen, and this ultimately affects the aquatic ecosystem. These toxic and harmful contaminants must be removed from textile wastewater to minimize the extent of pollution caused (or avoid causing pollution) when wastewater mixes with river water or is reused for other industrial or agricultural processes [26]. Hence, different textile wastewater treatment processes are discussed in the subsequent sections of this manuscript.

1.2 Research status and existing problems of printing and dyeing wastewater treatment technology at home and abroad

In recent years, extensive research has been carried out on treating dyeing and weaving wastewater (Fig. 1-2). To date, considerable efforts have been made to remove organic dyes/pollutants from wastewater using various methods (chemical, physical, and biological). It has been reported that it is challenging to remove color following traditional treatment methods (e.g., ozonation-, bleaching-, hydrogen peroxide/UV-, and electrochemistry-based) as most textile dyes have complex aromatic molecular structures that make their degradation difficult [12]. These dyes are stable in the presence of light and oxidants. They can also withstand conditions of aerobic digestion. Therefore, developing a green and sustainable method is essential to treat textile wastewater effectively. Environmental scientists and engineers have focused on developing economically viable treatment methods.

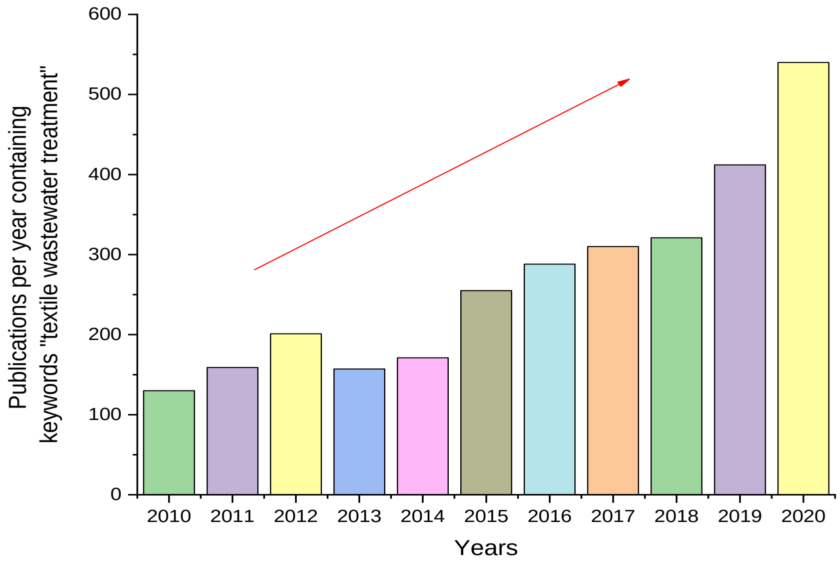

Figure 1-2. Several papers reported per annum (keywords used: "textile wastewater treatment") indexed in the core collection of Web of Science (spanning the years from 2010 to 2020 (inclusive); June 2021).

In the past two years, different treatment techniques have been studied to realize the sustainable degradation of textile wastewater (Fig. 1-3). More than 7×10^5 tons of dyes are synthesized annually worldwide. The structures of the dyes used in the textile industry are changed continuously to meet the color shade requirements and realize colorfastness [39]. The annual global market growth rate has been reported to be 8.13%. Hence, the textile industry will produce significant effluents containing approximately 280,000 tons of refractory textile dyes [41]. The choice of appropriate treatment method depends on the production process and the chemicals used in the textile plant. The choice is also influenced by the composition of wastewater, discharge rule, location, business costs, operational costs, availability of land, selection, and availability of reuse/recycling of treated effluents, process, and expertise [42]. The cost of treating river water used for drinking should be reduced, and drinking water should be colorless and devoid of toxic compounds. Thus, numerous treatment processes (such as physical, chemical, and biological) have been developed to treat wastewater before the wastewater is released into river bodies. Combining these treatment methods has also been used to treat textile wastewater economically and effectively. It has been reported that these techniques can be effectively used for textile wastewater treatment [2].

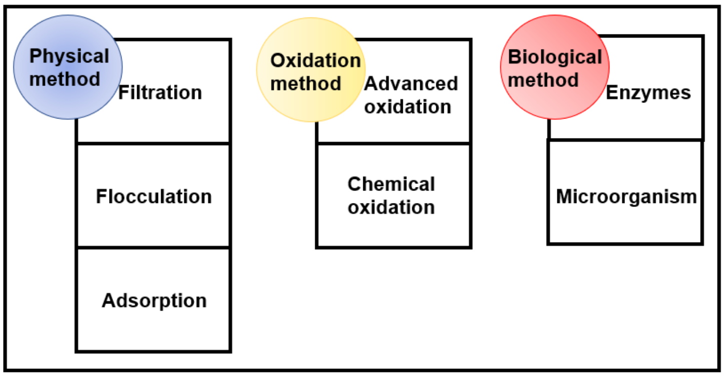

Figure 1-3. Treatment methods used for the degradation of dyes present in textile wastewater.

1.2.1 Physical method

Physical treatment methods involve the removal of substances from wastewater by exploiting commonly occurring forces (e.g., electrical attractive, gravitational, and van der Waals forces) or physical barriers. The use of these methods does not cause a change in the chemical structure of the substances present in water [10]. Occasionally, the physical state gets changed, or the coagulation of discharge substances takes place. The characteristics of some of these technologies have been explained in detail.

As one of the most widely used methods in the pretreatment of printing and dyeing wastewater, physical methods are further divided into adsorption, ion exchange, and membrane separation methods. The adsorption method mainly uses diatomite, activated carbon, and other adsorbents to adsorb various pollutants in the wastewater. The ion exchange method adsorbs the dyes of printing and dyeing wastewater to fixed exchange sites through ion exchange. The working principle of membrane separation technology is to use the selective permeability of the separation membrane to achieve the retention and recovery of pollutants in printing and dyeing wastewater. The physical method can usually achieve fast and efficient printing and dyeing wastewater treatment without secondary pollution. Still, there are also high equipment investment consumption, complicated operation, high cost of adsorbents and separation membranes, massive energy consumption, and the physical method does not realize dyes. Degradation increases the difficulty of subsequent processing.

1.2.1.1 Adsorption method

It is environmentally essential to remove dyes from colored effluents (especially from the effluents produced by the textile industries). The process of adsorption is one of the diverse methods that has been favorably used to treat dye-containing wastewater. A large number of materials, such as activated carbon (the most commonly used and expensive adsorbent), polymeric resins, and numerous economical adsorbents (agricultural and industrial by-products, such as peat, bentonite, chitin, silica, other clays, and fly ash) have been used as appropriate sorbents to realize the decolorization of industrial wastewater [43]. Activated carbon is the most well-known adsorbent. It is usually produced following the processes of physical or chemical activation. Although activated carbon can be effectively used to adsorb dyes, its application is limited as it is expensive. Hence, there is an increasing demand for producing an adsorbent as efficient as activated carbon but cheaper than activated carbon. Several economically viable treatment methods for the treatment of dyes have been reported. The execution of these methods requires the use of various adsorbents such as rice husk, cotton, bark, hair, coal, perlite, sewage sludge-based activated carbon, apple pomace, wheat straw, banana peel, orange peel, organobentonite, pearl millet husk, peat particles, wood, fly ash, and coal [44]. The removal ability of activated carbon processed from coir pith was studied in the presence of three strikingly applied reactive dyes in the textile industry [45]. It was reported that the activated carbon obtained from the coir pith effectively removed the color and significantly reduced the COD levels of textile effluents. Recently, Suleman et al. [46] used castor biomass-based biochar to realize the adsorption of safranin. The results revealed that the biochar could be used to realize a high adsorption capacity (4.98 mg/L). Approximately 99.6% of the safranin dye could be removed (99.6%). Oke and Mohan [47] reported that textile sludge-based activated carbon could be used as an adsorbent to adsorb reactive yellow 145, methylene blue, and reactive red 198. The adsorption capacities were recorded to be 75.1 mg/L, 101.8 mg/L, and 76.6 mg/L, respectively. It is noteworthy that removing activated carbon from the solution is challenging. Thus, it can be released into the environment and the processed sludge used to treat wastewater. This can result in secondary contamination [48]. Recently, titanium dioxide (TiO_2) adsorption is a highly regarded advanced photo-catalyst that constitutes a new growing branch of activated carbon composites to improve the adsorption rate and discoloration ability, causing severe consideration and support around the world [49]. Liu et al. fabricated a highly active TiO_2/activated carbon photocatalyst following a hydrothermal method. This system could be readily isolated from the treatment system and could be used to effectively adsorb methyl orange [50]. Activated carbon present in the TiO_2/activated carbon catalysts can potentially act as organic-molecule-adsorbing centers. Subsequently, the adsorbed molecules get transferred to the decomposition center, and the TiO_2 units present on the surface of activated carbon get illuminated. This can be attributed to the concentration differences (Fig. 1-4). It is essential to understand the conditions affecting the adsorption capability of such compounds. It is believed that the adsorption ability is influenced by various factors such as the hardness of water and the time of processing [51].

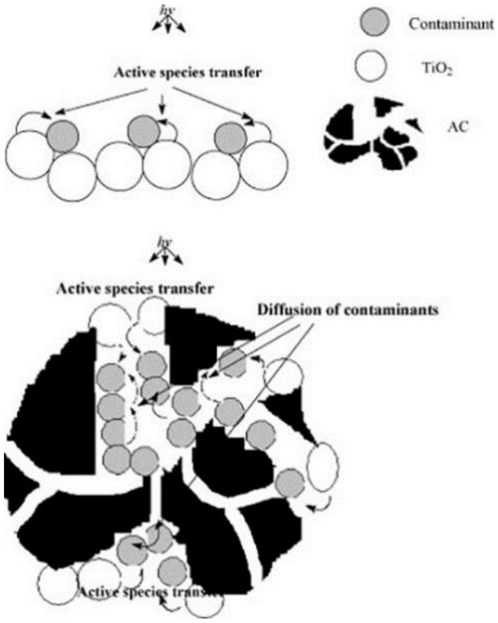

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Figure 1-4. Role of activated carbon in improving the activity of TiO_2; reproduced with permission from Ref. [50].

In general, the use of resin and activated carbon to adsorb and treat pollutants in printing and dyeing wastewater is the basic principle of adsorption, and its development is relatively mature. If the concentration of printing and dyeing wastewater is not high, activated carbon can be used for adsorption treatment, but it cannot meet the purpose of recycling. Its adsorption capacity will be affected by the pore size. Therefore, in future development, the research on activated carbon with large pore size will be the main focus to improve the treatment effect. Attention should be paid to the treatment of secondary pollution problems. Usually, solid-liquid separation is achieved using magnetic iron oxides, which helps complete recycling and dramatically reduces the cost of printing and dyeing wastewater treatment. Resin can also be used as an adsorbent, which has a better recovery capacity for chemical products; incredibly weakly basic ion exchange resins are widely used.

1.2.1.2 Coagulation and flocculation

Another method of treating textile printing and dyeing wastewater is coagulation and flocculation. Inorganic (aluminum, lime, and iron) and organic (polymer) coagulants can be used to remove the color from dyeing wastewater, either alone or in combination with each other [52]. Among them, alum is a valuable inorganic salt for removing dyes from textile wastewater [23]. Coagulation and filtration processes are also often combined to remove turbidity and discoloration-causing additives, such as dissolved organic carbon, iron (Fe), and manganese (Mn), while also eliminating heavy metals [53].

1.2.1.3 Membrane separation technique

Membrane separation technology is a technology that uses a special membrane to selectively separate components in a liquid. It is a high-efficiency separation, extraction, purification, and concentration technology with high separation efficiency, simple process, simple operation, effortless control, no secondary pollution, etc. According to the different pore sizes of the membrane, it can be generally divided into four categories, namely microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). Microfiltration can block the permeation of suspended solids such as bacteria, viruses, and large-scale colloids; ultrafiltration can remove macromolecular substances and particles in wastewater; nanofiltration can separate small molecular organic substances equal to water and inorganic salts; reverse osmosis only allows water molecules to pass through. The membrane separation technologies used to treat printing and dyeing wastewater are mainly ultrafiltration, nanofiltration, and reverse osmosis. Cheima et al. applied the ultrafiltration-nanofiltration double-membrane integrated process to the treatment of printing and dyeing wastewater. The research results show that the combination of nanofiltration and ultrafiltration increases the membrane flux, and the purification effect of printing and dyeing wastewater is enhanced [54].

The membrane filtration technique used to treat wastewater has attracted immense attention from people working in industries [55]. This technology can produce stable water without chemical consumption and low energy demand. What’s more, one of the most common methods is filtration technology. Textile industries have large amounts of coloring wastewater containing inorganic salts.

The filtration technology can be used to filter and recycle pigment-rich wastewater systems. This technique can also bleach and mercerize wastewater [18]. The process of membrane filtration proceeds over several steps. During NF, low-molecular-weight compounds and a few divalent salts are degraded (or broken into smaller units). The method of UF can be used to remove particles and macromolecules from the wastewater system, and the process of MF can be used to remove suspended matter [56].

The process of NF is being increasingly used to manage color emissions (Table 1-5). Tang et al. [57] followed the process of NF to treat textile wastewater to make the water reusable. The results revealed that high fluxes were generated under low pressures (≤500 kPa) conditions. The average dye removal rate achieved under these conditions was 98%, and the NaCl removal rate was less than 14%. Loose membranes, used to execute the NF process, are used in the art-of-the-state NF technology to achieve efficient fractionation of dyes and monovalent salts (i.e., NaCl). This is because the loose surface structure of the membranes facilitates the passage of salts [58]. A decrease in the penetration flux is observed during membrane processes, which can be attributed to the accumulation of molecules on the surface of the membranes. Noël et al. [59] have studied the efficiency of two types of membranes (NF45 and BQ01) using a direct dye solution. They conducted their experiments under conditions of an electric field to address the problem of fouling.

Recently, the process of UF has been used to recover high-molecular-weight and unsolvable dyes (such as indigo and dispersed dyes) [60]. The membrane used to execute the UF process is characterized by a porous structure. Moderate separation properties describe the process. The dimension of the membrane's aperture lies in the range of 2–200 nm. Thus, these membranes are more minor than large micro membranes but more significant than small nanofiltration membranes [55]. A low pressure-driven UF membrane was advanced using α-aminophosphonate-modified montmorillonite (MMT), cellulose acetate (CA), and Ag-TiO_2 nanoparticles to treat textile wastewater [26]. Jiang et al. [61] reported that the process of UF could be effectively used to realize dye/salt fractionation during the process of textile wastewater treatment. Barredo-Damas et al. [62] reported that the color removal efficiency varied between 82 and 98% when ceramic UF membranes were used.

The dimensions of the aperture of the MF membrane lie in the range of 0.2–0.5 μm. This membrane is primarily used to remove particulate suspensions and colloidal dyes from the load-running dye baths and out-of-date rinsing baths [63]. MF supports unconsumed auxiliary chemicals, dissolved organic pollutants, ions, and other soluble pollutions to pass through the membrane together with the permeability [64]. Amaral et al. [65] studied the use and operating parameters of the MF system to recover insoluble indigo blue present in the cotton yarn dye bath to realize pigment reuse and water washing. The results suggested that the MF process can potentially recover insoluble indigo blue presents in synthetic wastewater systems [65]. It has been recently reported that coal is a good carbon material that can be used as a carbon source to fabricate asymmetric microfiltration carbon membranes characterized by high porosity, controllable aperture, and narrow pore size [66]. A tubular carbon microfiltration membrane was fabricated by mixing mineral coal (average particle size: 100 μm) with a phenolic resin and organic additive [67]. The fabricated membrane exhibits exciting properties in terms of mechanical and chemical tolerance. In addition, high permeability flux and removal efficiency could be achieved. The extents of retention of COD values and salinity were 50% and 30%, respectively. Turbidity and color could be removed efficiently [67].

Table 1-5. Nanofiltration techniques are used to treat textile wastewater [55].

Dyes	Treatment condition	Removal rate (%)
Everzol black Everzol blue Everzol red	Initial concentration: 600 ppm. Pressure: 3–12 bars.	>90
Reactive black	Concentration: 0.4–2 ppm Pressure: 0.3–1.7 bars	60–97
Safranine orange	Initial concentration: 50 ppm Pressure: 5 bars	86
Eriochrome black	Initial concentration: 1 ppm Pressure: 4 bars	>99
Sunset yellow	Initial concentration: 100 ppm Pressure: 6.2–6.9 bars pH: 6.8	82.2

1.2.1.4 Magnetic separation technology

Magnetic separation technology uses a magnetic field to adsorb and separate different substances to reduce pollutant content. The pollutants in the printing and dyeing wastewater have other agglomeration properties. The magnetic separation technology uses paramagnetic and ferromagnetic particles to adsorb contaminants with a high degree of aggregation to separate and remove them. At the same time, "magnetic powder" is added to the printing and dyeing waste, and the paramagnetic and non-magnetic pollutants are adsorbed by the strength of the magnetic force. Then the separation technology is used for treatment [68].

1.2.1.5 Ultrasonic vibration method

The ultrasonic gas vibration method realizes the treatment of printing and dyeing wastewater by controlling the frequency of ultrasonic waves and saturated gas. The wastewater enters the air wave vibration chamber after adding the selected flocculant through the mediation tank. Under the intense vibration of a specific frequency, some organic substances in the wastewater are broken into small molecular substances. Under the accelerated thermal motion of water molecules, the flocculant rapidly coagulates. In the wastewater, the chroma, COD, aniline, etc., in the wastewater, will decrease accordingly, reducing the concentration of organic matter and realizing the treatment of printing and dyeing wastewater.

1.2.1.6 Reverse osmosis

The reverse osmosis (RO) technique can effectively remove mineral salts and organic compounds [56]. According to Jager et al. [69], RO impacts the residual salt and color significantly. RO membranes can remove inorganic ions and various combinations of organic molecules more efficiently than NF membranes. The infiltrates usually colorless and are characterized by low conductivity. These membranes should operate under high pressures and potential NF membranes alternatives. These can be used to recover wastewater from dyebath effluents [70]. Vishnu et al. [71] proposed a series of stained wastewater treatment methods, including NF and RO. They reported that these processes were economically and physically friendly. The functions could be effectively used to treat wastewater and recover salt. The wastewater treated using these techniques can be reused. The industry-specific RO treatment performance depends on the nature of the effluent and the pretreatment processes. Therefore, the outgoing water characteristics need to be evaluated before adopting specific RO methods. De Jager et al. [69] reported using a pilot-scale membrane bioreactor and a subsequent RO process. Although the effluent treated using the membrane bioreactor satisfied the discharge standard, the residual color and conducting wastewater needed to be separated following the RO process. Ebrahim et al. [72] used the RO technology to remove the acidic blue dye from industrial wastewater under various performing conditions (applied pressure: 5–10 bar; initial concentration of dye: 25–100 mg/L) to meet the concentration criteria laid down by the factory. The processes were conducted at a constant pH at room temperature. The results suggested that the removal efficiency increased during 90 min of the operation as the pressure raised to 98% and the original dye concentration [72].

1.2.2 Chemical process

The chemical method is a method of removing dyes by using chemistry or its theory. It mainly uses chemical reagents or electrical energy to remove or decolorize dyes in water, usually requiring specific chemical reagents or equipment. Chemical methods mainly include advanced oxidation and electrochemical oxidation. Most chemical processes require high costs, and the application of chemical reagents and the generation of intermediate products also bring about problems such as secondary pollution. Grčić et al. used the UV/Fe(II)/H_2O_2 advanced oxidation system to degrade the simulated printing and dyeing wastewater. Under the optimal conditions of adding 0.5 mM $FeSO_4 \cdot 7H_2O$ and 2.5 mM H_2O_2, the reaction was carried out for 60 min, and the TOC of acid blue 25 and medium orange 1 was removed. The treatment reached more than 90%, and the chroma removal rate was almost 100%, while the TOC removal rate for Direct Red 23 was only 34%, and the COD removal rate was only 48% [73].

In general, various chemicals are used during the execution of multiple processes to accelerate disinfecting wastewater during purification. These chemical processes involve different chemical reactions labeled as chemical unit processes. These processes accompany various biological and physical processes [10]. Conventional chemical methods (coagulation and flocculation), electrochemical techniques, and advanced oxidation processes are some of the methods and techniques that are commonly used to treat textile wastewater.

1.2.2.1 Traditional chemical methods

The coagulation/flocculation (CF) process is commonly used to destabilize particles or colloids. This process can be effectively used for dye removal [74]. The CF process is widely used to remove dyes as it is a cost-efficient process that is easy to operate [75]. Golob et al. [76] followed the CF method to purify residual dyebaths obtained when a cotton/polyamide blend was dyed black. During the CF process, selected coagulants play critical roles and help remove target contaminants. Various categories of coagulants (such as inorganic coagulants, inorganic-organic dual coagulants, and synthetic polymer flocculants) are commercially available [77]. Inorganic coagulants, such as iron and aluminum salts, are widely used in textile wastewater treatment [11]. Abbas et al. [78] reported that iron chloride (2.72 g/L) could be used to remove 91.89% of color attributable to the use of dyes. Recently, Jalal et al. [79] have reported using aluminum-based coagulants to treat textile wastewater. The maximum color reduction achieved was 98%. However, iron and aluminum salts, used as conventional coagulants, negatively impact the environment and human health. Environmentally-friendly substances that can be used as alternatives to these toxic coagulants have attracted the attention of scientists worldwide. Various biodegradable, non-toxic, and widely available compounds [80], such as chitosan, Moringa oleifera seeds, tannins, and Jatropha curcas seeds, are being studied [81].

1.2.2.2 Advanced oxidation processes

Advanced oxidation processes (AOPs), also known as deep oxidation technology, refer to the use of strongly oxidizing free radicals, ·OH to react with the macromolecular organic matter under particular experimental conditions (high temperature, high pressure, catalyst, etc.) structure, and degrade macromolecular organic matter into small molecular substances to achieve the purpose of oxidative removal, to realize the treatment of the organic matter. Advanced oxidation methods usually include several standard processes such as photocatalytic oxidation, ozone oxidation, and the Fenton method. Compared with ordinary oxidation methods, it has the following advantages: (1) the existing technology is challenging to deal with printing and dyeing wastewater, and advanced oxidation technology can directly mineralize dyes to achieve removal; (2) advanced oxidation technology The required reaction conditions are not high, no high temperature and high pressure are required, and it can be operated at room temperature and normal pressure; (3) the advanced oxidation process unit can be used alone, or in combination with other processes; (4) The catalyst consumption is low, and will not produce sludge. In practical applications, advanced oxidation technology has a better effect on macromolecular organic compounds that are difficult to oxidize in printing and dyeing wastewater [82].

In general, AOPs are up-and-coming alternatives that can be used to obtain hydroxyl radicals (HO·). These processes can effectively remove dyes and refractory contaminants [83]. AOPs include a series of methods, such as ozonation, photocatalysis, Fenton reaction, and Fenton-like processes [84]. Different types of AOPs, producing HO· are being increasingly studied to realize the de-coloration of textile effluents. The high reactivity of HO· can be attributed to the presence of unpaired electrons, and these radicals can help oxidize stubborn organic matter [85]. HO· is a nonselective and potent oxidizing agent. The rate constant recorded for the reactions (involving HO·) associated with removing organic matter lies in 109–1010 M^{-1} S^{-1} [86]. Besides, other oxidants such as sulfate ($SO_4^{\cdot-}$) radicals, permanganate (MnO_4^-), hypochlorite (ClO^-), chlorine dioxide (ClO_2), and ozone (O^3) are used during the process of textile wastewater treatment [87]. Khatri et al. [88] followed various AOPs based on zero-valent aluminum (ZVAl) to treat textile wastewater (Fig. 1-5). They reported that the maximum COD, color, and ammonia-nitrogen removal efficiencies achieved following the ZVAl-based AOPs were 97.9%, 94.4%, and 58.3%, respectively (conditions: ZVAl (1 g/L), Fe^{3+} (0.5 g/L), hydrogen peroxide (H_2O_2; 6.7 g/L), 3 h after contact time) [88]. Results obtained following the external addition of tert-butyl indicated that in situ HO· and SO4·- are the primary oxidants responsible for the oxidation of contaminants [88].

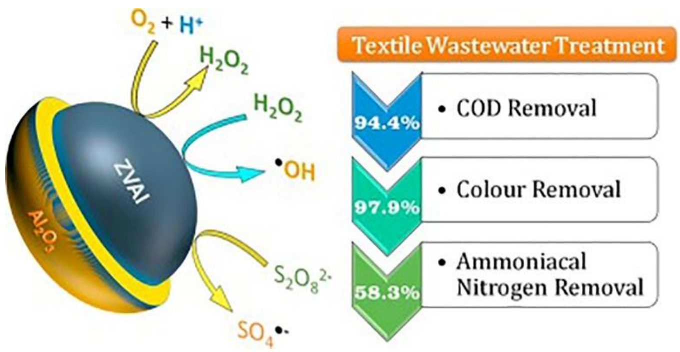

Figure 1-5. AOPs based on zero-valent aluminum for treating textile wastewater; reproduced with

permission from Ref. [88].

In recent years, ozone oxidation technology is an AOPs technology that has been widely used in the treatment of printing and dyeing wastewater. Ozone is a light blue gas with a pungent odor, which can not only directly and slowly oxidize organic matter in water, but also decompose under the action of alkali to generate active ·OH and react with organic matter, so that the chromophore in the dye can react with organic matter. The unsaturated bonds are broken to form colorless, small molecular weight aldehydes and organic acids, etc., degrading organic matter and decolorizing. Ozone oxidation technology has the advantages of less land occupation, simple reactor, easy to realize automatic control, no secondary pollution, and high decolorization efficiency and organic matter removal rate. Studies have shown that the composite oxidant combined with O_3 and H_2O_2 can increase the decomposition rate of O_3 and improve the treatment level. However, the solubility of ozone in water is negligible, and the utilization rate is low, which makes its consumption significant and the treatment cost high. Therefore, this method is not suitable for large-flow wastewater treatment.

Fe^{2+} and H_2O_2 are collectively called Fenton reagents. The Fenton method uses H_2O_2 to generate highly reactive OH under the action of Fe^{2+}, which has no oxidation selectivity and interacts with most organic matter to achieve the purpose of pollutant degradation. The Fenton oxidation method has been widely studied due to its advantages of simple operation, mild conditions, wide application range, and fast degradation rate. In the decolorization treatment of dyestuffs, H_2O_2 is a frequently used oxidant. When H_2O_2 is used alone, its oxidizing ability is weak. When it coexists with Fe^{2+}, its oxidizing ability is enhanced. Moreover, since Fe^{2+} has both coagulation effects, Fenton reagent hurts the effect of Fenton reagent on dyes in wastewater. Removal is very efficient [89]. Nonetheless, the Fenton oxidation process produces sludge, which requires subsequent treatment such as filtration.

The Electro–Fenton (EF) process combines various electrochemical methods and the Fenton oxidation process. The contaminant degradation process involves the Fenton reaction in solution (Eq. 1) and the direct oxidation of the substances on the anode surface (Eq. 2) [90]. During the EF process, H_2O_2 is continuously generated in solution following the oxygen reduction process by the cathode under acidic conditions during electrolysis (Eq. 3). Furthermore, in this process, the production of iron sludge is reduced by reproducing Fe^{2+} ions (Eq. 4) at cathode electroreduction Fe^{3+} [91]. The effectiveness of the EF technology in treating biologically-treated textile wastewater using graphite electrodes was investigated by Kuleyin et al. [91]. They reported that COD could be decreased by 93%, and 89% of the color could be removed when the concentration of Fe^{2+} was 2 mM, and the current intensity was 1.65 A [91].

$$Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+OH^{-}+HO^{\cdot} \quad (1)$$

$$M+H_2O \rightarrow M(OH^{\cdot})+H^{+}+e^{-} \quad (2)$$

$$O_2+2H^{+}+2e^{-} \rightarrow H_2O_2 \quad (3)$$

$$Fe^{3+}+e^{-} \rightarrow Fe^{2+} \quad (4)$$

The removal of color has been widely achieved using H_2O_2, sodium hypochlorite (NaClO), and other chemical agents in the textile industry. Argun and Karastas et al. [92] used 2000 mg/L of H_2O_2 to degrade synthetic dyes (concentration: 200 mg/L). However, the polishing operations associated with these chemicals are cost-ineffective [93]. It has been recently reported that the process of ozonation can be a potential alternative to established methods that are used for decoloration. According to Hassaan et al. [94], the removal of COD and ozone is much less efficient. It was observed that the process of ozonation could not be used to reduce COD effectively. The results revealed that it could be used to realize one-step decolorization and partial oxidation to improve biodegradability. The increased BOD/COD ratio following ozonation could be attributed to the increased biodegradability of toxic substances [94]. In addition, it has been suggested that O_3 should be used in combination with other technologies for effective wastewater treatment. According to Destaillats et al. [95], 30% mineralization of methyl orange can be achieved in the presence of O_3. When the process is conducted in combination with the ultrasound-based treatment methods, the cumulative extent of mineralization achieved is >80% in the pH range of 5.5–6.5. According to Vecitis et al. [96], the generation of the synergetic effect can be attributed to the decomposition of one O3 molecule resulting in the production of two $OH\cdot$ radicals under conditions of Sonolysis.

Another commonly used advanced oxidation method is photocatalysis. The photocatalytic oxidation method uses the energy generated under illumination to promote the transition of the energy level of the catalyst or oxide, and the resulting free radicals or empty orbitals have oxidizing solid properties. It can undergo a redox reaction with organic pollutants in wastewater to achieve the purpose of removing contaminants. Inorganic photocatalysts such as metal oxides, sulfides, and nitrides have received extensive attention from researchers. TiO_2 and ZnO can be used as photocatalysts to generate electron-hole pairs and free radicals to achieve the degradation of dye molecules. They are the two most widely studied catalysts at present. For example, the removal rates of reactive red 141 and ofloxacin by natural product-encapsulated ZnO prepared by Chankhanittha et al. were 100% and 98%, respectively, showing broad application prospects in the field of environmental remediation [97]. However, many photocatalysts in the current research are not sensitive enough to natural light, so it is imperative to develop photocatalysts with apparent responses to natural light.

In general, the commonly used chemical methods are the TiO_2-UV method, H_2O_2-UV method, O_3-UV method, etc.

1.2.2.3 Electrochemical process

Electrochemical treatment of printing and dyeing wastewater mainly includes micro-electrolysis, electrodialysis, and electrosorption. In the micro-electrolysis method, iron filings and carbon form a primary battery. The pollutants are chemically reacted on the electrodes, coupled with the electric enrichment of the primary battery itself, to remove the contaminants. The electrochemical oxidation method makes the pollutants undergo redox reactions at the cathode and anode to produce precipitates or gases. The principle of electrodialysis technology mainly uses the selective permeability of ion exchange membranes to separate electrolytes. The principle of the electro-adsorption method is the same as that of the adsorption mentioned above method. Still, the adsorption capacity of the adsorbent is increased, and the secondary pollution is reduced by electro-technology.

Electrochemical oxidation methods (e.g., electrokinetic coagulation, electroflotation, electrodecantation, electrooxidation, etc.) have recently emerged as the primary textile wastewater purification methods [17]. The primary reagents are electrons which are also labeled as the “clean reagents.” When electrons are used as the reagents, organic matters are generated, and secondary contaminants or by-products are not formed [98]. The electrochemical oxidation of C.I. acid orange 7 was performed on a boron-mixed diamond electrode [99]. The electrochemical treatment of the outflow from the UASB reactor (containing acid orange 7) promoted the reduction of aromatic amines. Reduction reactions were facilitated even when the concentrations were low, and the electrolyte already presents in the outflowing system was used [99]. Sakalis et al. [100] reported a new device that could be used for the electrochemical treatment of textile wastewater. The results revealed that the dye removal rate was 94.4% when the device was used to treat wastewater under conditions of neutral pH [100]. In addition to the management parameters, the rate of pollutant degradation is also influenced by the anode materials. Naumczyk et al. [101] demonstrated that several anode materials, such as graphite and precious metal anodes, can be successfully used to oxidize organic contaminants. Sakalis et al. [100] reported that the electrochemical treatment method is a relatively new method that can be used to achieve complete decoloration. The treatment methods can be conducted under conditions of medium pH. A low final temperature can be maintained, and the COD and BOD_5 values can be significantly reduced. The formation of sludge can also be avoided under these conditions. Unfortunately, in most cases, high concentrations of supporting electrolytes, particularly NaCl, are required to obtain acceptable results. This results in large amounts of free chlorine, hypochlorite anions, and polychlorinated aromatic products, significantly harming the environment [102].

1.2.2.4 Wet air oxidation process

Wet air oxidation is an advanced treatment method for high-concentration industrial wastewater successfully researched and developed in Japan in the mid-1980s. A process in which organic compounds and inorganic reducing substances in wastewater are oxidized into carbon dioxide and water in the liquid phase.

1.2.3 Biological process

The biological method uses the growth and metabolic function of microorganisms to absorb, decompose, transform, and absorb pollutants to separate contaminants from water bodies. The biological method is a classic method of sewage treatment. It has been developed for hundreds of years since applying the biofilm method, and various process technologies such as AAO, oxidation ditch, and SBR have been born. The advantages of this method are that it has little impact on the environment, and the price is low. The disadvantages are the slow reproduction speed of functional bacteria, the extended operation period, and the fluctuating treatment effect. Therefore, it is often combined with other methods to improve the efficiency of wastewater treatment. It is worth noting that biological decolorization proceeds in two ways, namely biosorption, and biodegradation. The mechanism of biosorption is mainly to use functional groups such as hydroxyl, phosphate, carboxylate, and amino on the cell wall of microorganisms to adsorb dye pollutants on the surface of microorganisms. Biodegradation is through the growth and metabolism of microorganisms, the chromophore bond is broken, and the dye is converted into an inorganic compound with relatively low toxicity to achieve the purpose of removing color. Biological methods are mainly divided into aerobic methods, anaerobic methods, and combined processes.

1.2.3.1 Aerobic method

The aerobic method has the advantages of mature technology and stable operation. Still, the disadvantage is that the gas explosion load and nutrient demand are large, and the processing cost is high. Commonly used suitable methods include ordinary activated sludge, circulating activated sludge, biological contact oxidation, sequencing batch bioreactor, etc. The activated sludge process is the most widely used physical process [103]. In this method, the air is continuously blown into the sewage in the detonation tank. After some time, the floccules with many aerobic microorganisms in the water—activated sludge—adsorb, physiologically metabolize and flocculate the organic matter in the wastewater, degrading organic matter. There are various forms of activated sludge methods. It is widely explored worldwide to increase the sludge concentration in the aeration tank and develop new gas explosion technology and solid-liquid separation technology to reduce power consumption, shorten treatment time and improve treatment efficiency. Compared with the activated sludge method, the biofilm method, which takes the tower biological filter and the contact oxidation of the physical film as the primary application forms, avoids the sludge bulking phenomenon, improves the treatment efficiency, saves the floor space, and has the shock fluctuation of the load has the characteristics of strong adaptability. However, although a single aerobic technology is used to treat printing and dyeing wastewater, although the BOD removal effect is noticeable, the removal rate of COD and chroma is not high, which is not conducive to subsequent processing.

1.2.3.2 Anaerobic method

The advantages of the anaerobic method are high organic load, low nutrient demand, low sludge bulking capacity, and strong toxicity resistance. At the same time, the disadvantages are poor wastewater treatment effect and long incubation time. At present, new anaerobic methods include anoxic baffled reactors, up-flow sludge beds and expanded granular sludge beds. Georgiou G et al. showed that the two-phase anaerobic fixed bed reactor could quickly remove the color of printing and dyeing wastewater and improve the biochemical properties of sewage. Appropriate carbon sources and sulfides to wastewater facilitate color removal [104]. Kim et al. investigated the effect of reducing agents on the treatment of azo dyes in anaerobic reactors and found that under HRT of 48h, intermittent addition of sulfide (mass concentration of 10 mg/L) could increase the decolorization rate of dyes by 9% [105].

1.2.3.3 Aerobic-anaerobic combined treatment

With the invention and utilization of new auxiliaries and dyes, the biodegradability of printing and dyeing wastewater has become increasingly poor. Researchers at home and abroad have begun to use combined processes to treat wastewater. Currently, aerobic-anaerobic combined treatment is the core of multi-stage treatment. Because aerobic-anaerobic collaborative treatment technology takes full advantage of anaerobic and aerobic biological treatment technology, it has become a research and application hotspot in the world. D.H. Shih et al. used MBBR (sequential anaerobic, anoxic, aerobic) to degrade high-concentration printing and dyeing wastewater, and the removal rates of COD and chroma could reach 85% and 70%, respectively [106]. Kapdan and Alprslan used a combined system of anaerobic filter and activated sludge tank to investigate the removal of COD and chroma from printing and dyeing wastewater under different HRT (12-72h) and different influent COD concentrations (800-3000 mg/L). Effect. The results showed that when the HRT was 48 h, the COD removal rate and decolorization rate reached 90% and 85%, respectively [107].

In general, the process of biological degradation of dyes is a green technology that can be used for removing dyes from textile wastewater. The cost of operation is minimum, and the process can be conducted under conditions of an optimal operating time. Ali recommends using biomaterials such as algae, bacteria, fungi, and yeasts (that can decompose and absorb various synthetic dyes) to achieve biological degradation [2]. The potential of multiple microorganisms to decolorize and degrade these harmful dyes has been reported (Table 1-6). We have discussed the processes of microbial decolorization and enzyme degradation in the following sections.

Table 1-6. Diverse aerobic bacteria can be used to achieve dye decolorization.

Azo dye	Microorganisms	Decolorization %	Ref.
Reactive Blue 13	*Proteus mirabilis LAG*	84	[108]
Methyl orange	*Kocuriarosea (MTCC 1532)*	100	[109]
Reactive orange 13	*Alcaligenes faecalis PMS-1*	100	[110]
Reactive blue 19	*Enterobacter sp. F NCIM 5545*	90	[111]
Reactive orange 16	*Microbial consortium DAS*	100	[112]
Blue Bezaktiv S-GLD 150	*Novel microbial consortium "Bx"*	88-97	[113]
Bacterial consortia*	Four individual azo dyes**	80-96	[114]
Bacterial consortium***	Red 198 azo dye	99.26	[115]

**Bacillus vallismortis*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*.

**Congo red, Bordeaux, Ranocid fast blue, and Blue BCC.

******Enterococcus faecalis-Klebsiella variicoa***

The biological wastewater treatment technique is technically attractive, environmentally friendly, and cost-effective [33]. Various bacteria can effectively remove dyes from wastewater as they can effectively discolor different dyes under anaerobic or aerobic conditions [116]. Attempts were made (as early as 1970) to identify bacteria that could degrade azo dyes. Three strains of bacteria were identified: *Bacillus subtilis*, *Aeromonas hydrophila*, and *Bacillus cereus* [117]. Recently, Spagni et al. [118] studied the applicability of immersed anaerobic membrane bioreactors in the decolorization of azo dye-containing wastewater. The results revealed that submerged anaerobic membrane bioreactors could be used to achieve a significantly high decolorization rate (>99%). It was also observed that *P. aeruginosa* could be used to remove commercially used tannery and textile dye. Navitan Fast blue S5R is an example of such a dye that can be removed from wastewater systems in the presence of glucose under aerobic conditions [119]. In addition, an anaerobic (facultative anaerobic bacterial culture)–aerobic sequence system was used to remove the color from a pilot-scale existing textile wastewater system and reduce the COD of the wastewater system under study [107].

The effect of microbial decolorization is influenced by the adaptability and activity of the chosen microorganisms [28]. From this point of view, in microbial decolorization, the bacteria can decolorize, which is assumed to be associated with the production of a different enzyme [28]. *Aeromonas veronii* GRI (KF964486) was isolated from domestic textile effluents after selective enrichment on azo dye to evaluate the biodegradation effectiveness of methyl orange [120]. It was observed that when the system was vaccinated with an initial light density of approximately 0.5, sucrose (0.25%), yeast extracts (0.125%), and SPB1 biosurfactant (0.01%), bacteria could effectively decolorize azo dyes. The stirring stage was initiated approximately 24 h after the stage of static incubation [28]. The researchers also reported that the removal of methyl orange could be potentially attributed to intracellular enzyme activities [28]. Furthermore, the textile dye-decolorization effect achieved in the presence of microorganisms could be improved by producing biological surfactants. Mnif et al. [121] reported that lipopeptides derived from *Bacillus subtilis* SPB1 maximize the decolorization efficiency and accelerate the decolorization rate when the optimal concentrations of biosurfactants are approximately 0.075%. It is essential to summarize the influence of each parameter associated with biodegradation to develop a more effective and faster bacterial degradation method. Table 1-7 summarizes the possible ranges of the staffing parameters to achieve better biodegradation effects [10].

Table 1-7. Differing factors affect the dye degradation and decolorization efficiencies [10, 122].

Factors	Descriptions
pH	The pH value has an essential effect on the dye decolorization efficiency. The optimal pH range for color removal using bacteria is 6.0–10.0. Tolerance toward high pH conditions is necessary for industrial applications where reactive azo dyes are used. Decolorization processes (for these types of dyes) are usually conducted under alkaline conditions.
Temperature	Temperature significantly influences all processes associated with microbial vitality. Water and soil repair processes are also affected by a change in temperature. The azo dye decolorization rate increases till the optimal temperature is reached. Following this, the decolorization activity decreases slightly.

Dye concentration	It has been previously reported that an increase in the dye concentration can gradually decrease the decolorization rate. This can be potentially attributed to the toxic effect of dyes exerted on the bacteria, inadequate biomass concentration, and blockage of azo reductase active sites in the presence of different dye molecules.
Electron donor	It has been observed that the addition of electron donors, such as glucose or acetate ions, can induce reduction cracking in azo bonds. The type and availability of electron donors significantly influence the color removal efficiency achieved using bioreactors operated under anaerobic conditions.
Oxygen and agitation	Environmental conditions can directly affect the degradation and decolorization process of azo dyes. The environmental reduction or oxidation states indirectly affect the operation of microbial metabolism. It is assumed that under anaerobic conditions, reductive enzyme activities are high. The oxidative enzymes involved with the process of azo dyes degradation require the presence of a small amount of oxygen.
Carbon and nitrogen sources	Dyes lack carbon and nitrogen sources. In the absence of a complementary source, it is difficult to biodegrade these dyes. For effective activity, microbial cultures often require the presence of complex organic sources and carbohydrates for efficient dye decolorization and degradation.
Dye structure	High rates of color removal were observed for dyes characterized by simple structures and low molecular weights. This could be attributed to the presence of electron-withdrawing groups (such as SO_3H and $-SO_2NH_2$) in the para position of the phenyl ring (relative to the azo bond) and high molecular weight dyes.
Redox mediator	Redox mediators (RM) can promote various reductive processes (including azo dye reduction) under anaerobic conditions.

Azo dyes are electron-deficient compounds containing the -N=N- chromophore group. These dyes may also have several other electron-withdrawing groups in their skeletal structure. The production of electronic defects makes the compounds less susceptible to the degradation process. Bacteria can effectively degrade dyes as diverse and well-constructed enzyme systems are present in these organisms [123]. It has been reported that azo dye-decolorizing microorganisms produce various enzymes, such as azo reductase, laccase, peroxidases, NADH-DCIP reductase, tyrosinase, MG reductase, and aminopyrine *N*-demethylase. Azoreductases, laccases, and peroxidases are the major enzymes responsible for the decolorization of azo dyes [124].

1.2.4 Possible combinations of different treatment methods

Most of the organic matter in sewage systems is non-biodegradable. This results in inefficient biotreatment. Chemical treatment technologies, such as flocculation and coagulation, can be used to remove color effectively. However, large amounts of harmful residues that require further treatment are produced when these techniques are used. This makes these technologies cost-ineffective [125]. Hence, various treatment methods have been suggested to treat wastewater effectively.

The CF and NF methods and a combination of the two methods (CF–NF) have been used to treat wastewater systems containing synthetic dyes [11]. Liang et al. [11] reported that the CF process could be used to achieve almost 90% dye removal efficiency under conditions of optimal dosage (polyaluminum chloride (PAC)/polydiallyldimethyl ammonium chloride (PDDA) = 400/200 ppm; pH of the mixed dye wastewater >3). In addition, they found that the CF and NF methods could complement each other's strengths. The problems faced when each technique was used individually could be addressed using the CF–NF method. Riera-Torres et al. [75] reported that NF removed except 40 and 80% of color for the five dyes, while CF reached 85 to 95% color removal rates of the four dyes except polyurethane resin for RB5. The color removal efficiency for RB5 reached 90%. When the combination techniques were used, the efficiency reached >98% for all dyes. Aouni et al. [126] reported that the color removal efficiency was >99% when the NF technique was used following electrocoagulation.

López-López et al. [127] improved the efficiency of AOPs by introducing CF as a pretreatment method (to reduce the turbidity observed in the textile effluents). The experiments were conducted with five different concentrations of industrial coagulants. The coagulants (FLOCUSOL-PA/18) were used to reduce turbidity (approximately 99% of the turbidity could be removed). In addition, the color removal rate for all AOPs was nearly 100%. Ozone combinations are the most widely used advanced oxidation methods before biological treatment to improve biodegradability and color removal efficiency. It has been reported that an increase in the BOD/COD ratio (following ozonation) can be attributed to the increased biodegradability of toxic substances [128]. According to Ledakowicz et al. [129], AOPs should be conducted before subjecting the water systems to degradation conditions. The results revealed that the combination of O_3 and UV radiation processes or the O_3/UV/H_2O_2 process were the highly efficient AOPs. The AOPs suppressed only 10% of the microbial growth (during the subsequent biodegradation process), while untreated wastewater exhibited 47% inhibition.

Microbial fuel cells (MFCs) have received immense attention as they can be efficiently used for power generation. These can also conduct sustainable wastewater treatment methods [130]. Electrochemically active bacteria (present at the anode) can produce electrons and reach the cathode via an external circuit. The formed protons and electrons bind with oxygen (in the cathode chamber) to make water. Recently, MFCs have been used to treat textile wastewater. Logroño et al. [131] designed an air-exposed single-chamber MFC with microalgal biocathodes to treat real-dye textile wastewater and generate bioelectricity. They reported high COD removal efficiencies (92–98%) using MFCs. Wu et al. [132] developed an innovative device that combined dual MFC to continuously remove Victoria Blue R and power production. The results revealed that when artificial wastewater containing 1000 mg/L of Victoria Blue R was continuously injected into the system, the Victoria Blue R removal rate reached 98.7%.

1.2.5 Challenges and future prospects

Although physicochemical methods are primarily used to treat wastewater, these methods lack versatility. The methods are cost-ineffective and less effective. Various wastewater components interfere with the treatment process, limiting the practical applications of these methods. The microbial decolorization method is economical and environmentally friendly. It is being increasingly used to treat textile wastewater. It is challenging to decolorize systems that contain complex and synthetic dyes. In summary, each method has its advantages and disadvantages (Table 1-8).

Table 1-8. Factors to be considered while choosing the treatment techniques for textile wastewater [19].

Treatment methods		Benefits	Factors to be considered
Physical method	● Adsorption	● Efficient removal of various types of dyes.	● Requires the use of regenerable or disposable adsorbents.

	• Membrane filtration	• Efficient removal of all types of dyes	• Production of concentrated sludge. Execution and running costs to be considered. Large volumes of waste cannot be treated.
Chemical method	• Chemical coagulation and flocculation	• Short detention time and high removal efficiencies for various types of dyes	• High cost of chemicals and reagents. pH needs to be adjusted. Large amounts of produced. Problems faced during handling and disposal of wastes.
	• Fenton oxidation	• Efficient removal for various types of dyes within a short period.	• Costly chemical reagents used. Large amounts of sludge produced. Problems faced during handling and disposal of wastes.
	• Ozonation	• Efficient removal for various types of dyes. • Sludge not produced.	• Not suitable for dispersed dyes. Short half-life (20 min).
	• Photocatalytic or sonocatalytic oxidation	• Efficient removal for various types of dyes.	• Relatively new methods. Formation of by-products observed. The penetrability of UV light (recorded or an aqueous medium) is often less than that of ultra-sound irradiation.
	• Radiolysis	• Complete mineralization is achieved in a short time. Sludge not produced. • Efficient removal for various types of dyes (at low volumes).	• Suitable for high concentrations of dyes. Relatively new method.
Biological method	• Aerobic process	• Efficient removal of azo dyes. • Low operating cost. Sludge can be recycled.	• Long detention times. Sensitive toward toxic organic dye.
	• Anaerobic process	• Low operating costs and biogas produced can be	• Cell reproduction stays longer. Sensitive to toxic organic dyes and the production of aromatic

	used as a fuel source.	amines.

Further studies should be conducted to develop ways of treating textile wastewater [2]. Combinations of various processes (such as AOP and biological combination processes) should be considered. The cost of the wastewater treatment method should be borne in mind while treating large amounts of wastewater. When the integrated processes (chemical and biological oxidation) were used for wastewater treatment, the costs increased when the reagent doses were raised at the Fenton reaction stage [133]. According to Holkar et al. [2] and Rodrigues et al. [133], the mineralization rates should be minimized during the pre-treatment or post-treatment phase to reduce unnecessary compounds and energy consumption usage and production. This will eventually help reduce the operation cost of the treatment processes.

1.3 Research background of landfill leachate

1.3.1 Hazards of landfill leachate

Landfill leachate is the microbial degradation and compaction of garbage in landfilling. Various components in it seep out with its water, and at the same time, it merges with the rainwater outside to form a pungent rancid odor and color—deeper high-concentration organic wastewater [134]. Landfill leachate is an integral part of environmental pollution. If not properly controlled and treated, it will cause serious harm to soil and water bodies, seriously harm the health of residents, and destroy the ecological balance. Specifically, some toxic and harmful substances in landfill leachate, such as heavy metals and high-concentration inorganic salts, will directly change the composition of the soil, significantly reduce soil fertility, cause soil salinization, and reduce crop yields. Under precipitation, toxic and harmful substances accumulated on the surface and soil will eventually be washed away or enter rivers, lakes, and other waters with rainwater runoff, destroying normal water body functions and the survival and reproduction of aquatic organisms. When humans eat crops or marine animals grown from polluted soil or water, it will cause harm to their health, and may lead to "three causes" effects in severe cases. It can be seen that the treatment of landfill leachate has crucial social value.

1.3.2 Characteristics of landfill leachate

It is estimated that 1 ton of solid waste will produce approximately 0.05–0.2 tons of leachate during the landfill life [135]. The main characteristics of landfill leachate are (Table 1): 1) complex composition, high concentration of organic matter and high metal content; 2) dark brown or black color, pungent odor; 3) high toxicity and poor biodegradability, COD (1000~5000 mg) /L) and ammonia nitrogen concentration are high, and BOD/COD is generally less than 0.1, the water quality fluctuates wildly; 4) The salinity is high, and the conductivity is as high as 20000~50000 μS/cm.

The composition of landfill leachate is complex, mainly: 1) organic substances, such as alcohols, aldehydes, short-chain sugars, various aromatic compounds, and humic acids; 2) inorganic substances, such as ammonia, sulfate, chloride ions; 3) heavy metal ions, such as Pb, Ni, Cu, Mn, Cr, etc. Landfill leachate has specific toxicity, significantly impacting its treatment process, especially the biological process [136]. Under the new standard's requirements, landfill leachate treatment technology demand is particularly urgent.

The water quality characteristics of landfill leachate are closely related to its age. It is usually divided into three stages: early (<5 years), middle (5-10 years), and late (>10 years). The value of BOD/COD is related to the age of landfill leachate. Most representatively, as the leachate ages, the BOD/COD gradually decreases, the leachate usually contains high concentrations of organic matter, and the early leachate has a high BOD/COD value (0.5-1.0). In advanced leachates, COD concentrations are below 4000 mg/L, BOD/COD ratios are low (< 0.1), other parameters such as ammonia nitrogen tend to increase with age, and pH increases.

Table 1-9. Main contaminants in landfill leachate [137].

Type	Concentration (mg/L)
Macro organics (dissolved organic matter)	COD: $0.0014\text{-}1.52\times10^5$
	BOD_5: $0.001\text{-}5.7\times10^4$
Macro inorganics (nitrogen compounds and salts)	NH_4^+-N: $0.05\text{-}2.5\times10^3$
	Cl^-: $0.03\text{-}4.5\times10^3$
	HCO_3^-: $0.61\text{-}7.3\times10^3$
	SO_4^{2-}: $0.008\text{-}7.8\times10^3$

Trace inorganics (heavy metals)	Cd: 0-0.4
	Cr: 0.02-1.5
	Cu: 0.001-10
	Pb: 0.001-5
	Ni: 0.015-13
	Zn: 0.03-1000
Trace organics	POPs: <1

1.4 Research status and existing problems of landfill leachate treatment technology at home and abroad

In general, the commonly used treatment technologies for landfill leachate include physical-chemical methods and biological treatment methods [138]. The physical and chemical treatment method aims to the advanced treatment of the organic matter in landfill leachate to improve biodegradability. Commonly used physical and chemical treatment techniques include adsorption, membrane separation, chemical precipitation, advanced oxidation, coagulation/flocculation, and other methods—combination process. For example, coagulation/flocculation can treat stabilized landfill leachate and is widely used in pretreatment steps. Commonly used coagulants are aluminum sulfate, ferric sulfate, ferric chloride, etc. Multiple coagulant combinations or coagulants added together with flocculants can improve the performance of flocculation and sedimentation, and the treatment effect is better. However, this treatment method also has some disadvantages, such as a large amount of sludge produced and the possible increase of aluminum or iron concentration in the effluent. The adsorption method uses the ability of the porous solid surface to absorb dissolved and colloidal substances in water. When the wastewater is in contact with the porous solid, some importance in the wastewater will be absorbed by the porous solid surface to treat wastewater. Commonly used adsorbents are activated carbon, zeolite, fly ash, and municipal waste incineration slag. In sewage pretreatment, adsorption is mainly used to remove a small number of heavy metal ions and refractory biodegradable organic matter, decolorization and deodorization, etc. However, due to the high concentration of pollutants in the leachate, this method is usually economical and practical for advanced treatment. Electrochemical oxidation is when macromolecular organic substances in waste liquid are oxidized into H_2O_2 and CO_2 by using vital oxidizing substances produced by anode catalytic ionization after electrification. Electrochemical oxidation can efficiently degrade high-concentration and macromolecular toxic and harmful substances in wastewater and improve biodegradability.

Compared with physical and chemical methods, biological treatment technology has significant advantages such as high treatment efficiency, no secondary pollution, high economic benefits, and low energy consumption. Biological treatment technology is mainly composed of anaerobic treatment technology, aerobic treatment technology, and various treatment methods that combine the two. According to the water quality characteristics of landfill leachate in different periods, the optimal treatment plan is selected to achieve the best treatment effect. Constructed wetlands, biological turntables, activated sludge methods, anaerobic fixed membrane bioreactors, and up-flow anaerobic sludge beds are all commonly used biological processes. The activated sludge method is widely used because of its low cost and high efficiency. Among them, the biological treatment method based on activated sludge has the advantages of low operating cost, good treatment effect, and no secondary pollution. It is widely used in landfill leachate treatment. Activated sludge is rich in microbial flora, which can use the pollutants in landfill leachate as a growth substrate to induce enzymes to assimilate, maintain biomass, achieve the transformation and decomposition of pollutants, and achieve the purpose of purifying sewage. Many scholars have found that traditional activated sludge can remove more than 80% of the organic carbon and 99% of BOD5 in the leachate. Even if the organic carbon in the influent is as high as 1000 mg/L, the sludge biological phase can quickly adapt and degrade role [139]. In addition, M.EI-Fadel et al. studied the treatment of high-strength landfill leachate with membrane bioreactors (MBRs) such as flat plate (FS) and hollow fiber (HF) at different solid residence times (SRT=5~20 d). efficiency and membrane fouling [140]. Using the 16S rDNA gene sequence analysis method, the bacterial community of the mixed solution was tested for a long time, trying to determine the relationship between the treatment effect of the membrane reactor and the composition of the microbial community. The results showed that the removal efficiencies of the two membrane systems were comparable, with a significant drop in removal efficiency as the SRT decreased to 5 days. Ammonia and nitrite-oxidizing bacteria were not detected at the set solids residence time of the experiment.

1.5 Analysis of the research status of modern microbial technology

As people's requirements for environmental quality continue to increase and improve, the requirements for wastewater discharge standards are becoming more and more stringent. In the biological treatment of wastewater, the activated sludge method is still the most widely used treatment technology globally, but it produces a large amount of excess sludge at the same time as sewage treatment, and now there are secondary methods for the disposal of excess sludge. The problem of pollution or high cost, the reduction technology of excess sludge is a hot field of research for environmental protection workers. Biological treatment of printing and dyeing wastewater refers to the use of activated sludge or functional microorganisms to destroy chromophoric groups or sulfonic acid groups and other auxiliary color groups to achieve the degradation of dyes and other pollutants. Therefore, the key to biodegradation is the selection of efficient decolorizing functional microorganisms. At present, many organisms have been proved to have the ability to degrade dyes, mainly including fungi, bacteria, and algae. Since the 1970s, Horitsu et al. isolated the first strains capable of degrading azo dyes. Since then, there have been more and more reports on dye-degrading bacteria. Cai et al. isolated Acinetobacter sp. SRL8 from sludge and the strain could decolorize 90.2% of 300 mg/L azo dye. Disperse Orange 30 within 48 h under microaerobic conditions at 30 °C and pH 7.0 [141]. Proreus mirabilis screened by Mohanty et al. can decolorize 95.0% of 100 mg/L vat green 1 within 20 h under aerobic conditions [142]. Khataee et al. found that Cladophora glaucescens can degrade a variety of azo dyes using the produced azoreductase (AZR) [143]. Bacteria, fungi, and algae can only degrade one or one type of dyes in wastewater treatment. The dye concentration and decolorization spectrum are all low, so it is difficult to achieve large-scale application in complex printing and dyeing wastewater, and mixed bacteria can pass through. The synergy between microorganisms significantly improves the decolorization efficiency of dyes. On the other hand, degrading enzymes rich in microbial flora can decolorize various types of high-concentration dyes and achieve complete degradation of intermediate metabolites, which has more practical application value.

1.6 Metagenomic sequencing

In the era of high-throughput functional genomics, the research on microbial community ecology has been dramatically developed, mainly due to DNA sequencing technology's progress, enabling researchers to explore the composition and function of microbial communities in a high resolution and independent of pure culture [144]. Like amplicon sequencing, metagenomic sequencing is also a non-culturable microbial community analysis method. But it makes up for the limitation that amplicon sequencing cannot examine many microbial genes in a short time. The metagenomics used the bird-gun method to randomly break all the genetic materials in the microbial community into millions of gene fragments. The initial short sequence (read) obtained by sequencing was assembled into a long line (contig) through multiple assembly and then further assembled into a scaffold for gene identification. At present, metagenomic technology has been applied to the study of different microbial groups, involving a wide range of natural environments, small to artificial environments, and even humans.

1.7 Novel Vertical Flow Labyrinth (VFL) Device

Vertical Flow Labyrinth (VFL, Vertical Flow Labyrinth) technology originated in Slovakia, Europe, in 1990 (Figure 1-6). In 1992, this technology was applied to build the first small sewage treatment station. The technology inventor, Mr. JURAJ, initially used it for the life of single-family residents. Sewage treatment, because this technology is suitable for the scattered living of European families and the characteristics of point drainage, that is, the situation of scattered sewage discharge and huge fluctuation, it technically solves the problem of impact resistance and stable operation of small sewage treatment facilities, and It can be fully automatic and unattended [145].

Figure 1-6. Vertical Flow Labyrinth (VFL).

After the technology matures, it gradually develops to a larger scale, such as the processing scale of dozens of tons in small villages and small communities, the processing scale of several hundred tons in sports fields, commercial buildings, and tourist areas, and the processing scale of several thousand tons and tens of thousands of tons in small towns. The family-type integrated equipment that can process 1 ton of water per day has developed into a large-scale reinforced concrete combined pool with a daily processing capacity of 10 million tons (Figure 1-7).

Figure 1-7. Scale-up vertical Flow Labyrinth (VFL).

The new and efficient biological treatment device constructed by VFL technology combines anaerobic, anoxic, and aerobic organically to create the most favorable physiological activity environment for the microorganisms required for sewage treatment and fully realize the degradation of organic matter nitrification and denitrification (Figure 1-8). The role of phosphorus accumulation is to determine the adequate volume of each area of the structure according to the incoming and outgoing water's water quality and control the gas explosion in the sludge return through the oxidation-reduction potential detection in the aerobic zone and then manage the entire system.

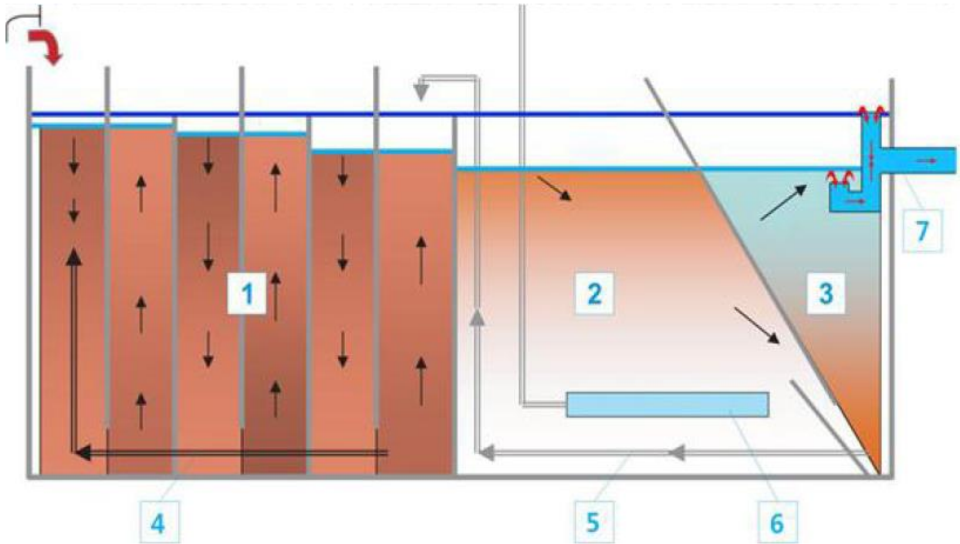

Figure 1-8. VFL reactor device; 1-anaerobic and anoxic zone; 2-aerobic zone; 3-settling zone; 4-internal circulation; 5-sludge return; 6-tubular microporous aerator; 7-drainage tank

As shown in the figure below (Figure 1-9), the combined tank is generally designed in two groups, symmetrical left, and right, and the long and narrow part in the middle of the tail of the integrated tank is the sludge tank. Depending on the amount of water, it can be determined to start a single group or a double group. The combined pool's anaerobic zone and anoxic zone are vertical flow labyrinths. The features are: the reactor has built-in vertical baffles, which divide the reactor into N series reaction chambers, each of which is a relatively independent up and downflow sludge bed system. The water flow is guided up and down by the deflector and passes through the sludge layer in the reaction chamber one by one. The substrate in the influent is fully contacted with the microorganisms to be degraded and removed. The front end of the combined zone is the anaerobic zone, and the sewage enters the anaerobic zone, the anoxic zone, the aerobic zone, the sedimentation zone, and the drum filter along with the deflector.

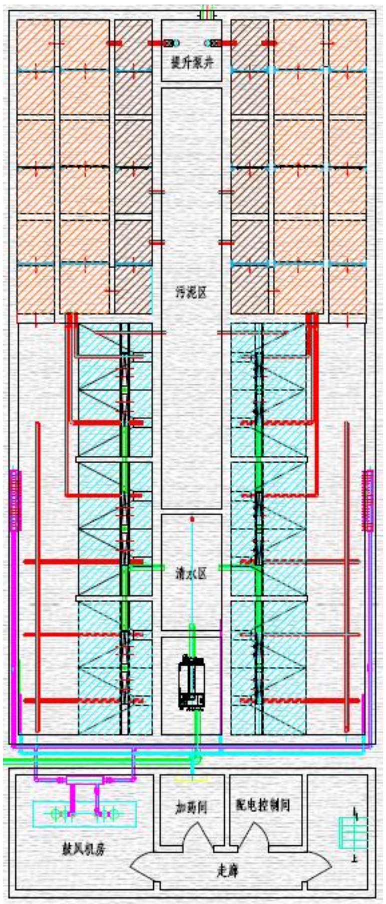

Figure 1-9. Schematic diagram of VFL reactor.

1.8 The source of the topic, the purpose and significance of the research

1.8.1 Research purpose

In recent years, with the development of my country's social economy and the improvement of people's living standards, people's requirements for the quality of water environment are getting higher and higher. On the one hand, it is difficult for the effluent of the traditional biochemical treatment process to meet the requirements of increasingly stringent water quality standards. On the other hand, the increasing shortage of water resources brought about by economic development also urgently requires the development of new and suitable wastewater recycling technologies to alleviate the tension between the supply and demand of water resources. As a new and improved high-efficiency sewage biological treatment technology, VFL is gradually applied to the recovery and reuse of sewage. Compared with the traditional aerobic reaction device, it is confirmed that VFL can improve the shock load resistance of the system, enhance the efficiency of biological nitrogen and phosphorus removal, and inhibit sludge bulking.

1.8.2 The main research content of the subject

Taking landfill leachate and sand washing wastewater as the research objects, using the new VFL device as the treatment method, and aiming at the reuse of urban sewage, the process mechanism, biodegradation characteristics, and response changes of sludge community of the VFL device for sewage purification were studied.

1.8.3 Analysis of Subject Innovation

- A new type of VFL device treatment method is proposed for high-concentration refractory landfill leachate and sand washing wastewater, and its long-term operation feasibility and stability are investigated;
- Changes in the microbial community structure in the new VFL device reveal the biological removal mechanism of pollutants.

1.8.4 Subject significance

To sum up, there are potential water quality safety problems in my country. Landfill leachate is an integral part of environmental pollution. If it is not adequately controlled and treated, it will cause serious harm to soil and water bodies, seriously harm the health of residents, and destroy the ecological balance. Specifically, some toxic and harmful substances in landfill leachate, such as heavy metals and high-concentration inorganic salts, will directly change the composition of the soil, significantly reduce soil fertility, cause soil salinization, and reduce crop yields. In addition, due to the significant changes in the quality and quantity of printing and dyeing wastewater and the continuous development and application of new processes, new raw materials, new dyes, and new auxiliaries, the wastewater pollutants discharged during the production process have become more and more complex, and the difficulty of treatment is also increasing. The effect of the original printing and dyeing wastewater treatment technology becomes worse. With the development of the textile printing and dyeing industry, the treatment of printing and dyeing wastewater has become one of the bottlenecks to reducing water pollution and achieving the healthy development of the industry.

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Chapter 2

EXPERIMENTAL MATERIALS

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2.1 Experimental installation

2.1.1 Process flow of new VFL device

The front end of the VFL device is the anaerobic zone, and the sewage enters the anaerobic zone, anoxic zone, aerobic zone, and the sedimentation zone successively along with the deflector (Fig. 2-1). The VFL technology process is in the anaerobic zone, where the water flows vertically. The reactor has a built-in vertical baffle, which divides the reactor into several reaction chambers in series. Each of which is a relatively independent up and downflow sludge bed system. It is equivalent to an up and down flow sludge treatment system, so the sludge concentration is relatively high in the anaerobic zone. Compared with the traditional sewage treatment plant with many tanks, all the processes of the VFL device are completed in one reaction tank. That is, anaerobic, anoxic, aerobic, including precipitation, can be built in a reaction zone to achieve.

The VFL combined tank used in this experiment is based on the principle of the activated sludge method and uses microbial degradation to remove organic pollutants in sewage. Advantage. The design scale is 15000 m^3/d, the design size is 52 m×73 m×5.7m, the adequate water depth is 5 m, and the practical volume is 18998 m^3. In addition, the residence time in the anaerobic acidification section of the VFL tank was 3.1 h, the residence time in the anoxic section of the VFL tank was 6.2 h, the residence time in the aerobic section of the VFL tank was 12.5 h, and the residence time in the sedimentation section of the VFL tank was 4.9 h.

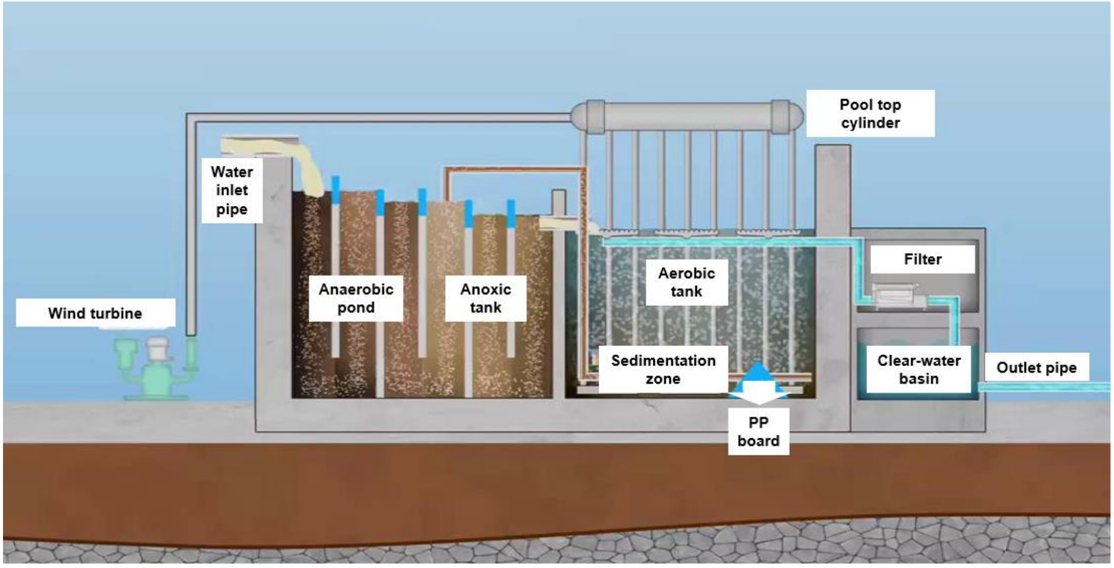

Figure 2-1. The VFL technology process.

2.1.2 AAO reactor

Anaerobic-Anoxic-Oxic (AAO) activated sludge method sets up the anaerobic tank, anoxic tank, and oxic tank in sequence in the sewage treatment process and realizes it through the return of sludge between the tanks [1, 2]. Simultaneous removal of organic pollutants, nitrogen, and phosphorus in wastewater [3-5], a typical AAO process flow is shown in Fig. 2-2.

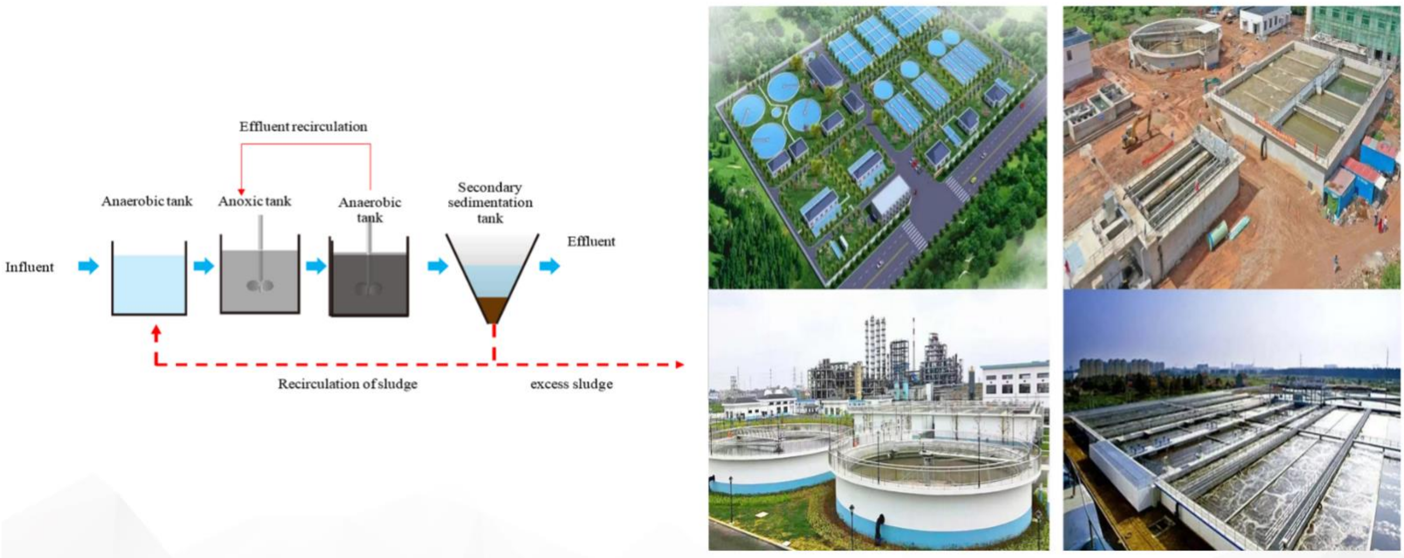

Figure 2-2. AAO process flow diagram.

2.2 Analysis of wastewater source and water quality characteristics

2.2.1 Beijing wastewater (landfill Leachate) source

The landfill leachate was obtained from Beijing Gaoantun Waste Incineration Co., Ltd.

2.2.2 Source of Huzhou wastewater (printing and dyeing wastewater)

The wastewater is taken from the large-scale effluent in the sewage treatment plant, and the wastewater is mainly sand washing wastewater and printing wastewater (Table 2-1).

Table 2-1. Influent water quality.

Pollutant name	pH	COD (mg/L)	BOD_5 (mg/L)	SS (mg/L)	NH_4^+-N	Chroma（times）	TDS（mg/L）
Influent water quality	6-9	≤800	≤300	≤500	≤50	≤200	≤2000

2.3 Routine experiment items and analysis methods

2.3.1 Conventional water quality indicators

Table 2-2. Quality indicators.

Items	Instrument and equipment
COD	5B-3B (V8)-Multi-parameter water quality analyzer
NO_2^--N	5B-3B (V8)-Multi-parameter water quality analyzer
NO_3^--N	5B-3B (V8)-Multi-parameter water quality analyzer
NH_4^+-N	5B-3B (V8)-Multi-parameter water quality analyzer
TP	5B-3B (V8)-Multi-parameter water quality analyzer
Temperature（℃）	S220 multi-parameter tester
pH	S220 multi-parameter tester
Conductivity（ms）	S220 multi-parameter tester
BOD_5	Dilution and inoculation method（HJ 505-2009）

2.3.2 Detection of VFA

Liquid sample pretreatment: take 1 mL of sample, add 200 μL of 50% sulfuric acid, add 100 μL of 1000 mg/L internal standard (cyclohexanone) solution and 2 mL of ether, homogenize for 1 min, centrifuge at 4 °C and 12000 rpm for 10 min, take the supernatant and test on the machine [6].

GC-MS detection method: Column: Agilent DB-WAX capillary column (30 m x 0.25 mm x 0.25 μm), the carrier gas is high-purity helium (purity not less than 99.999%), flow rate 1.0 mL/min, inlet temperature 220 °C, injection volume of 1 μL, splitless injection, and solvent delay time of 3.5 min [7-9].

Mass spectrometry system: electron impact ion source (EI), ion source temperature 230 ℃, interface temperature 220 ℃ [10, 11].

Table 2-3. Warming program.

Gradient temperature (℃)	Time (min)	Heating rate（℃/min）
40	3	30
210	5	

MS collects data in SIM mode, the acquisition interval is 0.3 s, and the target ions are shown in the following table:

Table 2-4. The target ions.

Numbering	Name	Target ion（m/z）
1	Acetic acid	60
2	Propionic acid	74
3	Isobutyric acid	73
4	Butyric acid	88
5	Isovaleric acid	60
6	Valeric acid	87
7	Caproic acid	60

2.3.3 Sludge index（MLSS，MLVSS，MLVSS/MLSS（%））

Mixed liquor suspended solids (MLSS) and composite liquid volatile suspended solids (MLVSS) are indicators to indirectly measure the microbial biomass of activated sludge [12-14]. MLSS represents the prime minister (mg/L) of activated sludge solids in the mixed solution per unit volume [15], and MLVSS refers to the volatile suspended solids of the hybrid solution [16]. Domestic sewage is generally MLVSS/MLSS=0.7 [17]. Measuring MLSS requires qualitative filter paper (can not use quantitative), electronic analytical balance, oven, dryer, etc. Take 100 mL of the mixture and filter it with filter paper. After the temperature in the range rises to the set value between 103-105 °C, put the filter paper after filtering into the oven to dry for 2 hours, then take it out and place it in a desiccator for half an hour. After weighing, subtract the weight of the filter paper, and use the same procedure as above to measure the importance of the filter paper [18, 19]. The experiment must strictly follow the above operation. Otherwise, there will be deviations.

Water sample collection, preservation, and precautions: The sampling location is set at the exit of the aeration tank; the water depth of the aeration tank is 3.1 meters, so sampling should be taken at 0.78 meters below the liquid surface, and the actual sampling location should be in the center of the sampling section.

Detection method: Evaporate the evenly mixed liquid on a steam bath or water bath in an evaporating dish weighed to constant weight, and place it in an oven at 103-105 °C to dry to stable weight. The added weight is MLSS. The sample for which MLSS has been measured is placed in a muffle furnace at 600°C and fired, and the reduced weight is MLVSS [20, 21].

2.4 Microbial morphology

The sludge morphology in the reactor was observed with an Olympus BX 61 optical microscope. The straightforward method is as follows: take an appropriate amount of sludge on a glass slide and spread the sludge evenly. After the sample is air-dried, place the sliding glass on the microscope stage and select a suitable objective lens for observation [22, 23].

2.5 Molecular biological analysis methods

Most microorganisms in the environment cannot achieve pure culture. The laboratory culture conditions cannot be wholly simulated to obtain consistent results with the microbial diversity in the activated sludge of the mixed culture system under the actual sewage treatment conditions. Metagenomics has received increasing attention in recent years. In 1998, the concept of metagenomics was first proposed by Handelsman et al. [24, 25]. Metagenomics is a kind of research object that takes the microbial population genome in environmental samples as the research object and uses the sequencing analysis of functional gene screening as the research method [26, 27]. A new approach to microbial research for research purposes [28]. With the development of high-throughput nucleic acid sequencing technology and bioinformatics, meta-omics technology has become one of the hot spots in microbiome research in sewage biological treatment systems. Regulatory mechanisms provide essential tools. Generally, in the study of metagenomics methods, the genomic DNA is first extracted from environmental samples, then cloned into a suitable vector, and then introduced into the host bacteria. Later, the target transformants are screened and sequenced (Fig. 2-3).

Figure 2-3. Metagenomics workflow.

2.5.1 Metagenome assembly and annotation for Illumina sequencing

Sample extracted from the reactor, DNA for 16S rRNA gene amplicon sequencing. The mass and concentration of each sample were measured using a Nano Photometer spectrophotometer and a Qubit 2.0 Flurometer. The Illumina sequencing library was constructed using the DNA Library Prep Kit, which mainly includes the following process: fragment the DNA sample to 350bp, and then complete the preparation of the entire sequencing library through the steps of end repair, adding sequence adapters, sample noise reduction, and PCR amplification, and finally using Illumina HisSeq platform performs metagenomic sequencing [29].

After processing the raw data with Readfq software, clean data was obtained, and the obtained valid sequences were de novo assembled, and MetaGeneMark was used to predict the open reading frame of the contigs generated after assembly. The microbial community information in the metagenome was determined by systematically calculating the sequence after BLAST alignment in the NCBI database combined with the LCA algorithm. The functional gene annotation of Genesets was aligned in the KEGG and eggNOG available databases using DIAMOND software [30, 31].

2.5.2 Genome binning based on metagenomic

For Illumina sequencing, genome binning was performed using MaxBin based on the frequencies of the four nucleotides in the sample and information on single-copy marker genes. The bin sequences were calculated using an expectation-maximization algorithm to determine genome size, GC content, genome integrity, and genome coverage levels. However, the completeness, contamination level, and strain heterogeneity of the binning-obtained draft genomes were assessed by checkM software. The available information of KEGG and eggNOG was annotated with DIMOND software according to the predicted CDS [32]. However, all annotation information in the resulting three bins was integrated using Circos. Finally, a phylogenetic tree of the resulting bins was constructed using Fasttree software.

2.6 Experimental Statistical Methods

Species and gene abundance data obtained by metagenomic sequencing can be further subjected to statistical analysis, including differential analysis, grouping and clustering, significance, and association analysis [33].

（1） Use STAMP to analyze the differences between groups on the normalized OTUs, species, and gene abundances.

（2） SPSS 12 was used to calculate the Pearson/Spearman correlation index.

（3） The Venny 2.1 tool was used for the Venn analysis.

（4） Use the online tool provided by KEGG (http://www.genome.jp/keeg/) to perform metabolic pathway analysis on the KEGG module (Module), the mapper (Mapper), and pathway (Pathway).

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Chapter 3

STUDY ON THE OPERATIONAL EFFICIENCY AND SLUDGE CHARACTERISTICS OF THE VERTICAL FLOW LABYRINTH (VFL) DEVICE FOR BEIJING LANDFILL LEACHATE TREATMENT

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3.1 STUDY ON THE OPERATIONAL EFFICIENCY OF THE VERTICAL FLOW LABYRINTH (VFL) DEVICE FOR BEIJING LANDFILL LEACHATE TREATMENT

In recent years, with the improvement of living conditions and the accelerated pace of urbanization, the generation and accumulation of urban waste has increased, and the original landfills and treatment plants have become increasingly saturated [1, 2]. With the promotion of garbage classification, garbage treatment plants have gradually attracted people's attention [3]. Landfill leachate comes from precipitation and high-concentration organic wastewater brought by the garbage itself [4]. Its composition is complex, with high biochemical oxygen demand and COD, high ammonia nitrogen content, and large changes in water quality and water quality [5, 6]. If it is not treated, it is directly discharged, which will cause serious pollution to the environment. In general, landfill leachate composition is complex, with a wide variety of organic matter and extremely poor biodegradability [7]. The organic matter components mainly include humic acids, fulvic acids, polycyclic aromatic hydrocarbons, and other refractory substances. Anaerobic biological treatment can effectively degrade COD in high-concentration organic wastewater, improve the biodegradability of sewage, and have low operating costs [8]. Previous studies have shown that anaerobic bioreactors such as UASB, IC, EGSB, and CLR can effectively dispose of landfill leachate [9, 10]. To solve the problems of poor effluent quality in the operation of a conventional anaerobic process for landfill leachate treatment, this chapter uses a VFL reaction device to treat landfill leachate and investigates the effect of landfill leachate load on the removal efficiency of the VFL process.

The emergence of the anaerobic-aerobic combined process is mainly based on the enhanced removal of pollutants by the anaerobic process [11]. For wastewater with a high concentration of ammonia nitrogen in the influent, the effective reduction of ammonia nitrogen cannot be achieved only by the anaerobic reactor, and even the accumulation of ammonia nitrogen will occur [12]. Compared with the anaerobic process, the advantage of the aerobic process is that the organic matter in the wastewater can be completely removed. From the perspective of energy consumption, the aerobic process needs to continuously explode to ensure the growth of microorganisms, increasing the amount of sewage. Therefore, the use of an anaerobic process before the aerobic process can significantly reduce the sewage load and improve the biodegradability of the sewage [13]. As an anaerobic-aerobic process, VFL has the incomparable advantages of a separate aerobic process and a different anaerobic process:

1) Since the anaerobic reactor removes a large amount of organic matter and suspended solids in the wastewater, the amount of sludge in the subsequent aerobic process will be much smaller, so the container will be much smaller.

2) The anaerobic reactor can save the operation unit required for sludge stabilization: the excess sludge in the aerobic part can be recycled to the anaerobic reactor, where it is nitrified and thickened.

3) The amount of excess sludge is much less than that of the aerobic process alone because the sludge yield in an anaerobic environment is much smaller than that in aerobic conditions. In addition, the sludge concentration of the anaerobic reactor is much higher, so it is easier to handle.

3.1.1 Chemical Oxygen Demand Removal Effect of Continuous Experiments

Chemical oxygen demand (COD) is a chemical measure of the amount of reducing substances in a water sample that need to be oxidized [14]. Oxygen equivalent of substances (usually organic) can be oxidized by strong oxidants in wastewater, wastewater treatment plant effluent and contaminated water [15]. It is an important and relatively rapid determination of organic pollution parameters [16]. As shown in Fig. 3-1, the experiment started on June 30, 2021, and continued to monitor COD every day until December 20, 2021. The VFL reaction unit started operation at a higher feed concentration, and the average COD concentration of the influent was 3772.6 mg/L. At day 58, COD showed an upward trend. The highest value of COD influent can reach 6460 mg/L, and the corresponding COD in the anaerobic section, anoxic section, aerobic section and effluent of the VFL unit on the same day reaches 1088 mg/L, 1670 mg/L, 1079 mg/L, and 872 mg/L, respectively. The COD concentration in the influent > the COD concentration in the anoxic stage > the COD concentration in the anaerobic stage > the COD concentration in the aerobic stage > the effluent COD concentration. For long-term (170 days) sampling and testing, the VFL device can effectively remove high COD concentration influent water, and the removal rate can reach up to 86.5%. The effluent COD was stable in the whole stage, and the concentration fluctuation of the influent did not affect the effluent COD concentration.

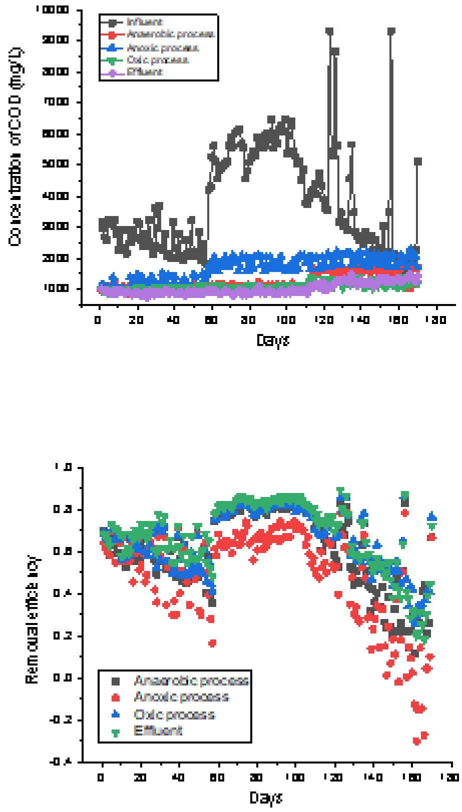

Figure 3-1. COD concentration and removal efficiency of VFL process over time.

In contrast, Zhang et al. treated landfill leachate using the electro/Fe^{2+}/peroxodisulfate process and showed that only 28.1% of the COD was removed [17]. Bashir et al. used electrochemical oxidation technology to treat landfill leachate (Table 3-1). The results showed that when the COD influent concentration was 1414 mg/L and the current density was 79.9 mA/cm^2, the COD removal rate reached 68% [18]. Xu et al. compared the performance of biological, chemical, and biochemical methods for treating landfill leachate [19]. The natural method uses an activated sludge anaerobic sequencing batch reactor, while the chemical process uses ozone (O_3), ozone plus hydrogen peroxide (O_3+H_2O_2), and Fenton reagent (H_2O_2+Fe^{2+}) and ozone plus Fenton reagent (O_3+H_2O_2+Fe^{2+}). The experimental results show that when the COD influent concentration reaches 1276 mg/L, the activated sludge anaerobic sequencing batch reactor can remove 25% of COD, ozone can effectively remove 52% of COD, and Fenton reagent can effectively remove 67% of COD. COD, while ozone combined with Fenton's reagent can effectively remove 72% of COD [19].

Table 3-1. Compared with other treatment processes.

Initial COD (mg/L)	Operational methods	Performance removal (%)	Reference
2000-4600	Aerobic reactors	46-64 COD	[20]
2900	Aerobic reactors	75 COD	[21]
3130	Aerobic reactors	69 COD	[22]
7439	Aerobic reactors	78-98 COD	[23]
5750	Aerobic reactors	62 COD	[24]
879-940	Granular activated carbon	91 COD	[25]
5295	Sequencing batch reactor	62 COD	[26]
1414	Electrochemical oxidation	68 COD	[18]

3.1.2 Effect of nitrogen removal

Nitrogen is one of the most important elements in nature. Nitrogen in the water mainly exists in the form of ions, mainly nitrate nitrogen (NO_3^-), nitrite nitrogen (NO_2^-), ammonia nitrogen (NH_4^+ and NH_3) and so on [27]. Nitrates themselves are not harmful to the human body. However, if a higher concentration of nitrate enters the human body and is converted into nitrite under the action of enzymes, nitrite has great harm to the human body [28, 29]. After nitrite enters the human body, it can be directly absorbed by the blood. Due to the oxidizing property of nitrite, it can convert hemoglobin into methemoglobin. The oxidized hemoglobin loses its oxygen transport function, resulting in human hypoxia, which affects the normal function of the human body, and can seriously cause nerve damage. systemic hypoxia or respiratory failure [30]. Nitrite is also an indirect carcinogen, which can combine with amines in the human body to form the "tri-to" substances nitrosamide and nitrosamine [31]. Biological nitrogen removal from wastewater, primarily composed of a combination of aerobic nitrification and anaerobic denitrification, is usually considered to accomplish optimal and economic nitrogen treatment (Fig. 3-2) [32].

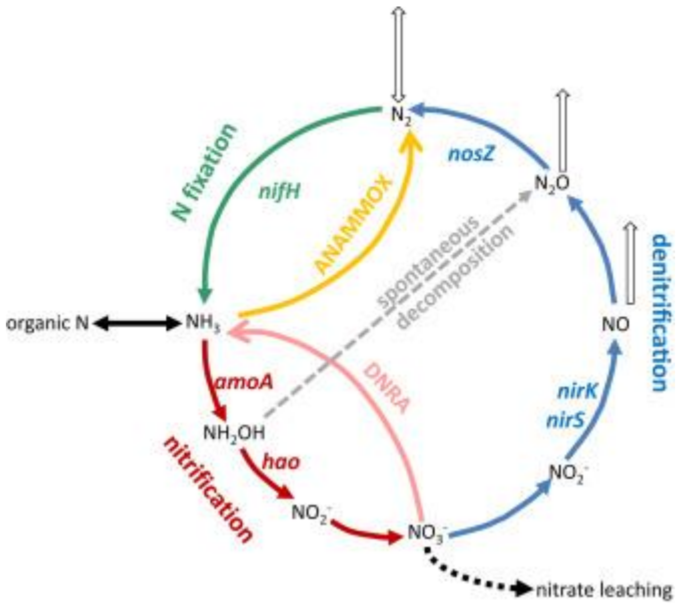

Figure 3-2. The biological N cycle.

Biological nitrogen removal is a highly valued nitrogen removal technology [33]. In the biological denitrification process, nitrogen transformation includes ammonification, nitrification and denitrification [34, 35]. As far as we know, ammoniation takes place under aerobic or anaerobic conditions, nitrification must react under aerobic conditions, and denitrification takes place under anaerobic conditions [36]. Specifically, nitrogen-containing inorganic or organic compounds are decomposed into ammonia nitrogen under the action of aerobic bacteria, and this process is called ammonification [37]. Through the action of nitrifying bacteria or nitrifying bacteria, ammonia nitrogen is converted into nitrate nitrogen or nitrite nitrogen, and this process is called nitrification [38, 39]. Under anoxic or anaerobic conditions, nitrate or nitrite nitrogen is reduced to nitrogen by denitrifying bacteria, which is called denitrification [40].

The NO_2^- removal effect of the VFL device in this experiment is shown in Fig. 3-3. Although the overall NO_2^- concentration of the reactor is not high, the concentration of NO_2^- effluent is higher than that of the influent. The average NO_2^- concentration of the effluent was 18.7 mg/L. In general, NO_2^-concentration in aerobic stage> anaerobic stage NO_2^- concentration> effluent NO_2^- concentration> anoxic stage NO_2^- concentration> influent NO_2^- concentration. Combined with the above analysis, our preliminary inference is that ammonia nitrogen is converted into nitrate nitrogen or nitrite nitrogen through the action of nitrifying bacteria or nitrosating bacteria.

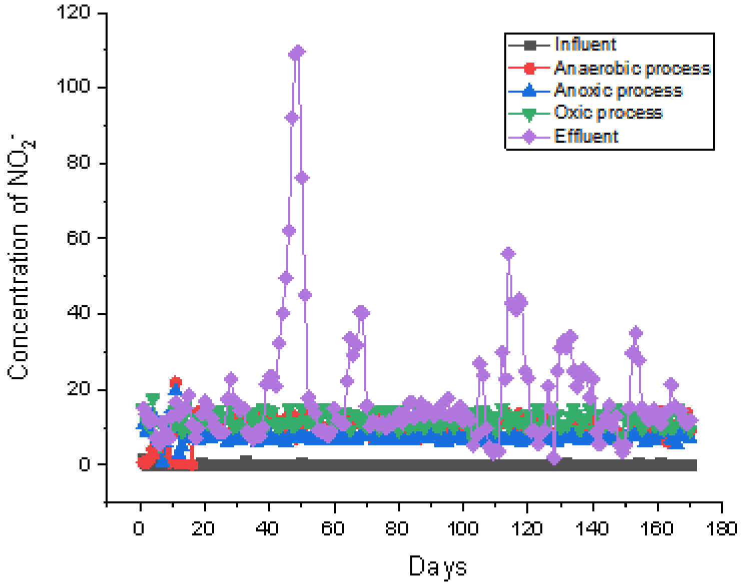

Figure 3-3. Operational effect of VFL process for nitrous over time.

The NO_3^- contained in wastewater is a major cause of water eutrophication and regional water quality deterioration [41, 42]. The converted NO_2^- into drinking water will also be toxic to the human body. Therefore, the removal of NO_3^- has always been an important aspect of water technology. link. The traditional biological removal of NO_3^- is to use the coordinated action of heterotrophic microorganisms denitrifying bacteria. Under anoxic and anaerobic conditions, when electrons are transferred from the donor (NO_3^- or NO_2^-) to the acceptor, the microorganisms obtain energy, using to maintain the life activities of existing cells and synthesize new cellular substances, this process converts NO_3^- in wastewater into N_xO and N_2 gases. can be expressed as:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

In nature, denitrifying bacteria are widely distributed, and the denitrification process is ubiquitous. In this experiment, the difference in NO_3^- concentration in the five stages is not huge, but in general, the effluent > aerobic stage > anoxic stage > anaerobic stage > influent. The average concentration of NO_3^- in the effluent was 22.6 mg/L, while the influent was only 5.0 mg/L (Fig. 3-4). Combined with the above analysis, our preliminary inference is that ammonia nitrogen is converted into nitrate nitrogen or nitrite nitrogen through the action of nitrifying bacteria or nitrosating bacteria. Kondaveeti et al. used a bioelectrochemical denitrification system to treat landfill leachate. Although the NO_3^- removal rate was between 81-97% when the applied voltage was 0.7-2V, the coulombic efficiency was reduced from 74% to 19% [43]. In another recent study, the microalgae *Chlorella vulgaris* was used to treat landfill leachate, and the study showed that the NO_3^- removal rate was only 60.9% with conventional photobioreactors [44]. Wu et al. used anammox post-denitrification technology based on partial denitrification to treat landfill leachate, and their experimental results showed that 11% of NO_3^- was produced [45].

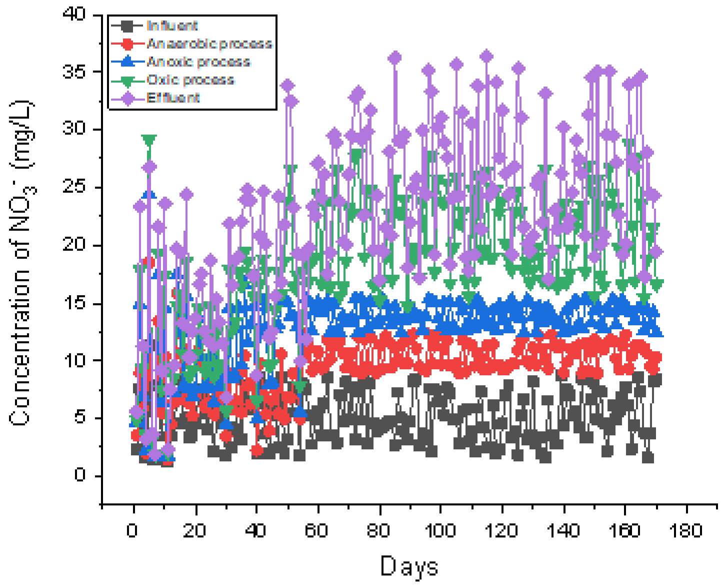

Figure 3-4. Operational effect of VFL process on nitrate over time

The high content of NH_4^+ in landfill leachate is one of the difficult problems in treatment [46, 47]. Biological denitrification can achieve the true meaning of nitrogen removal, rather than "pollution transfer", so biological denitrification is the most economical, effective and widely used method for the treatment of landfill leachate [48]. This experiment was taken from Beijing Gaoantun Waste Incineration Co., Ltd., and the NH_4^+ concentration was as high as 2348.5 mg/L (Fig. 3-5). The average concentration of NH_4^+ in the effluent is 15.5 mg/L, which meets the requirements of China's national secondary emission standard, where the NH_4^+ concentration is lower than 25 mg/L, and the NH_4^+ removal rate is as high as 99.3%. Wang et al. used a simultaneous partial nitrification, anammox and denitrification (SNAD) reaction unit to treat landfill leachate, and the results showed that the removal rate of NH_4^+ was as high as 98.9-99.9% [49]. Göçer et al. used an anaerobic folded plate reactor to treat landfill leachate. When the dilution rate of landfill leachate was 20%, the NH_4^+ removal rate was 5% [50]. Yalçuk et al. used a constructed wetland system to treat landfill leachate, and in their study, the removal rate of NH_4^+ was up to 60% [51]. Overall, the VFL unit operates excellently for NH_4^+ removal.

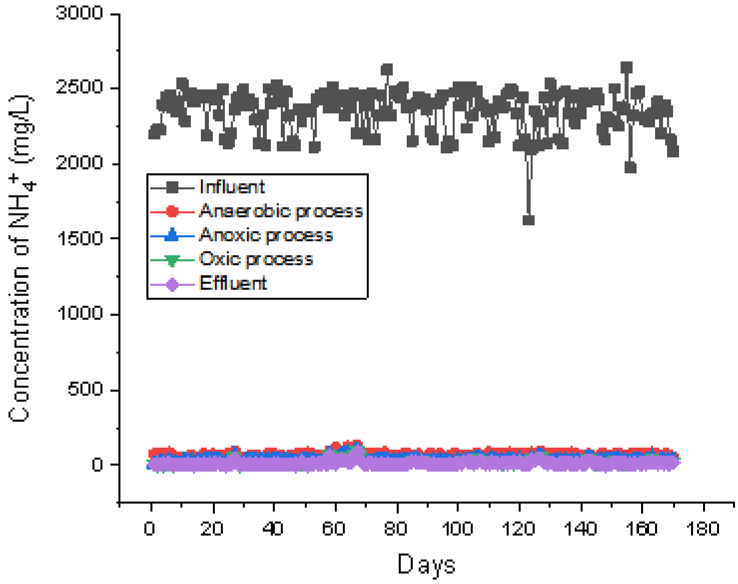

Figure 3-5. Ammonia removal effect of VFL process over time

As the main indicator of pollutants, total nitrogen is particularly important for evaluating the effect of pollutant removal. This experiment has a good removal effect on total nitrogen (97.1%). The average total nitrogen concentration in the influent was 2635.7 mg/L, while the average total nitrogen concentration in the effluent was 77.4 mg/L (Fig. 3-6). And the influent concentration > anaerobic section > anoxic section > effluent > aerobic section. The average total nitrogen concentration in the aerobic section was 72.2 mg/L. Combined with the above analysis, NO_3^- and NO_2^- affected the removal of total nitrogen. Song et al. used a low dissolved oxygen composite biological system to treat old landfill leachate, which consisted of an anaerobic biological turntable and four aeration tanks, and showed that the maximum total nitrogen removal rate was only 84.06% [52]. In general, in terms of nitrogen, the VFL unit has a significant degradation effect on landfill leachate.

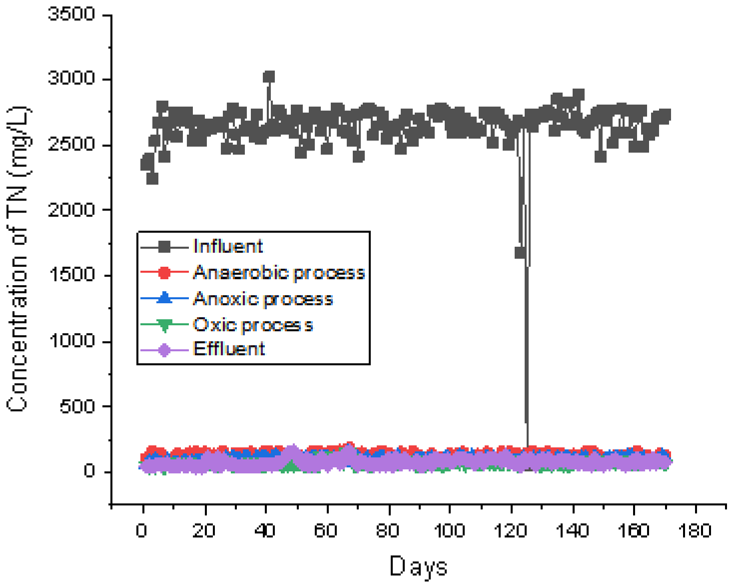

Figure 3-6. Total nitrogen removal effect of VFL process over time.

3.1.3 Total phosphorus removal

Phosphorus pollution can cause eutrophication of water bodies, resulting in a decline in water quality [53]. Generally speaking, if the total phosphorus in the water body exceeds 0.05 mg/L, the water body is considered to be in a state of mild eutrophication. The phosphorus concentration in the landfill leachate is generally between 3.5-16 mg/L. The calcium ion concentration and total alkalinity content in the landfill leachate are higher, which affects the phosphorus concentration in the leachate and makes the solubility of the leachate. Phosphate concentrations are lower. The total phosphorus concentration is 0-120 mg/L. Figure 3-7 shows the removal effect of VFL device on total phosphorus in landfill leachate. It can be seen from the figure that the degradation effect of total phosphorus is obvious. The total phosphorus concentration in each stage was relatively stable. The total phosphorus content in the influent water is high, at 10.3-15.7 mg/L. The total phosphorus content in the effluent is low, at 3.9-4.87 mg/L. Phosphorus removal is accomplished through the anaerobic release of large amounts of phosphorus by phosphorus accumulating bacteria, the absorption of large amounts of phosphorus under aerobic explosion conditions, and the final removal of sludge with high phosphorus content. The anaerobic release of phosphorus is the premise of aerobic absorption. The more sufficient the anaerobic phosphorus release is, the more favorable it is to absorb phosphorus by phosphorus accumulating bacteria, and the lower the phosphorus content in the effluent, the better the removal effect. Overall, the total phosphorus treated by the VFL unit was 67.2% lower than that of the influent. Phosphorus fluctuates greatly in the influent stage and the anaerobic stage. Hu et al. used *Chlorella vulgaris* and Scenedesmus dimorphus co-culture to remove landfill leachate, and the experimental results showed that the removal rate of total phosphorus could reach 86% [54].

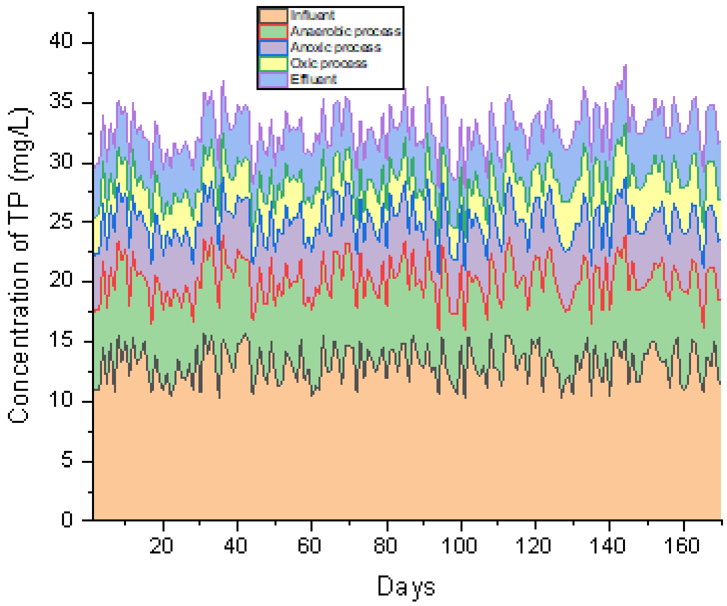

Figure 3-7. Total phosphorus removal effect of VFL process over time

3.1.4 Reaction unit temperature monitoring

From June 30, 2021 to December 20, 2021, the temperature showed a trend of first stabilizing and then decreasing (Fig. 3-8). The highest temperature was as high as 28.6 °C, while the lowest temperature was 10.9 °C. Low temperature has inhibitory effects on microbial reproduction and nitrification and denitrification reactions. Combined with the above basic water quality indicators (NO_2^-, NO_3^-, NH_4^+, COD, total phosphorus), the effect of temperature on its removal effect is not obvious.

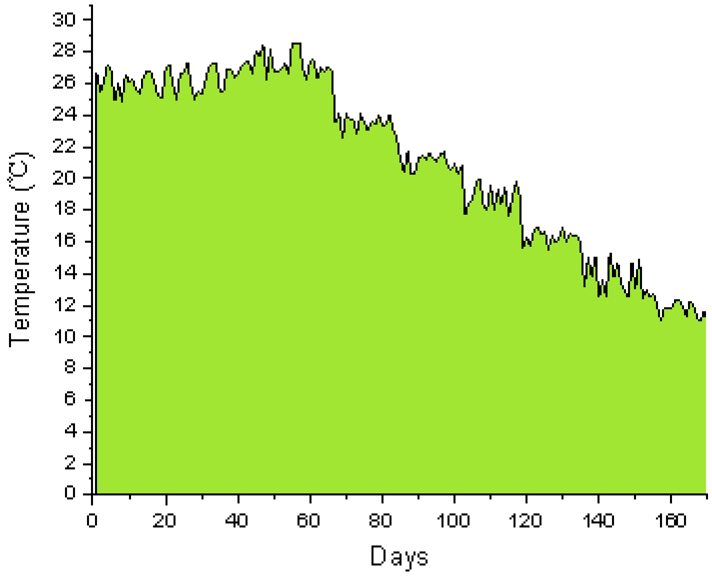

Figure 3-8. Temperature monitoring during VFL unit operation

3.1.5 Changes in wastewater pH, conductivity and VFA

Unsuitable pH will reduce the activity of enzymes, thereby affecting the biochemical processes of microorganisms [55]. The pH of landfill leachate is generally 4-9. The average pH of the influent is 7.8, the average pH of the outflow is 8.4, and the pH is slightly higher than that of the influent (Fig. 3-9). In addition, the average pH of the anaerobic, anoxic, and aerobic stages was 8.4, 8.3, and 8.5, respectively. The aerobic stage is slightly higher, which may be caused by the degradation of volatile fatty acids produced in the anaerobic stage of the system. In addition, it is inferred that ammonia nitrogen is gradually oxidized to nitrate, and the alkalinity is continuously consumed, causing the pH of the system to drop. The rise and fall are offset, so that the pH of the VFL device remains relatively stable. In addition, the above temperature change did not affect the pH change in the apparatus.

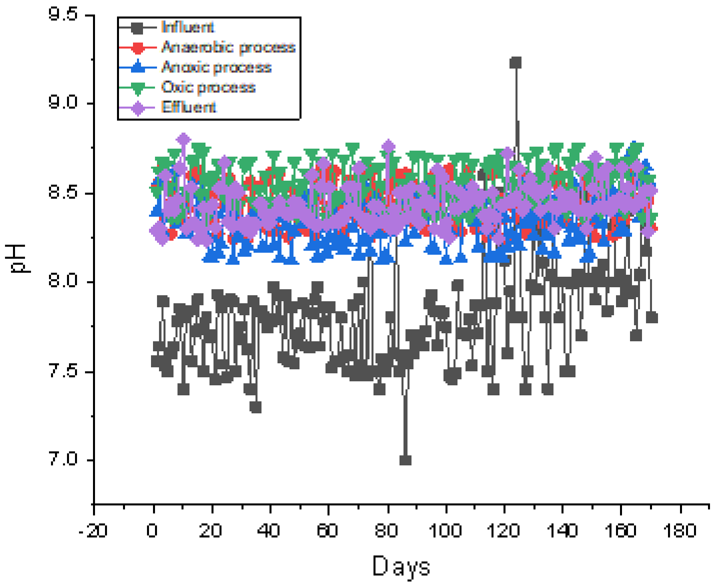

Figure 3-9. pH monitoring during VFL unit operation

Conductivity is a parameter used to describe the ease with which charges flow through a substance. The conductivity of water directly reflects the level of impurities in the water. Landfill leachate contains a relatively high concentration of total dissolved solids, and water-insoluble carbonates, metals and their metal oxides will dissolve, so the leachate contains many types and high concentrations of metal ions [56]. It has been reported that the conductivity of some landfill leachates has exceeded 40,000 µs/cm. The conductivity monitoring of the VFL under long-term operation is shown in Fig. 3-10. The conductivity of the influent water is significantly higher than other stages. Specifically, the average conductivity of the influent is 31.8 ms/cm, the average conductivity of the anaerobic section is 20.6 ms/cm, the average conductivity of the anoxic section is 19.4 ms/cm, and the average conductivity of the aerobic section is 19.9 ms/cm, the average conductivity of the effluent water was 21.1 ms/cm. The decrease in conductivity is presumed to be due to the oxidation of organic matter in the water to CO_2 and H_2O. In addition, high concentrations of inorganic soluble salts, iron, magnesium, etc. are absorbed and utilized by microorganisms in the matrix, so the electrical conductivity decreases.

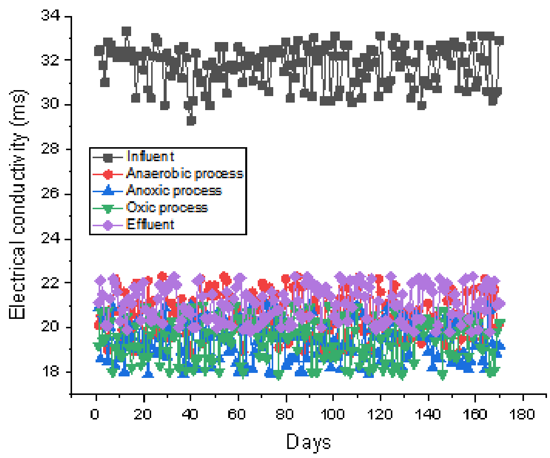

Figure 3-10. Conductivity monitoring during VFL unit operation.

Volatile fatty acids are important products during the oxidation of organic substrates by anaerobic microorganisms, and their concentrations can affect the performance of the reactor [57]. In addition, volatile fatty acids can also be used as carbon sources for nitrogen and phosphorus removal from wastewater, and the denitrification population prefers acetic acid, followed by butyric acid, and then propionic acid [58, 59]. Volatile fatty acid is an important indicator for evaluating landfill leachate, which not only affects the reaction process of anaerobic digestion of organic waste, but also directly affects the selection of leachate treatment process [60, 61]. Therefore, it is very necessary to study the components and proportions of volatile fatty acids in the analysis system. The volatile fatty acids monitored in this experiment include: acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid (Fig. 3-11). The highest concentration of acetic acid was detected in the influent, with an average concentration of 118.5 mg/L. The proportion of volatile fatty acids in the influent water is: acetic acid (118.5 mg/L)>propionic acid (4.27 mg/L)> isovaleric acid (3.57 mg/L)> isobutyric acid (3.36 mg/L)> Valeric acid (0.78 mg/L)> butyric acid (0.73 mg/L)> caproic acid (0.54 mg/L). At the same time, acetic acid is also a volatile fatty acid with the fastest consumption rate. It is worth noting that butyric acid, isobutyric acid, valeric acid, isovaleric acid and caproic acid were not detected in the anaerobic, anoxic, aerobic, and effluent sections. The concentration of acetic acid detected in the effluent was only 0.82 mg/L. The degradation rate of acetic acid in the whole VFL system reached 99.3%. It can be seen that it is speculated that volatile fatty acids can be used as carbon sources, and the VFL device can degrade volatile fatty acids.

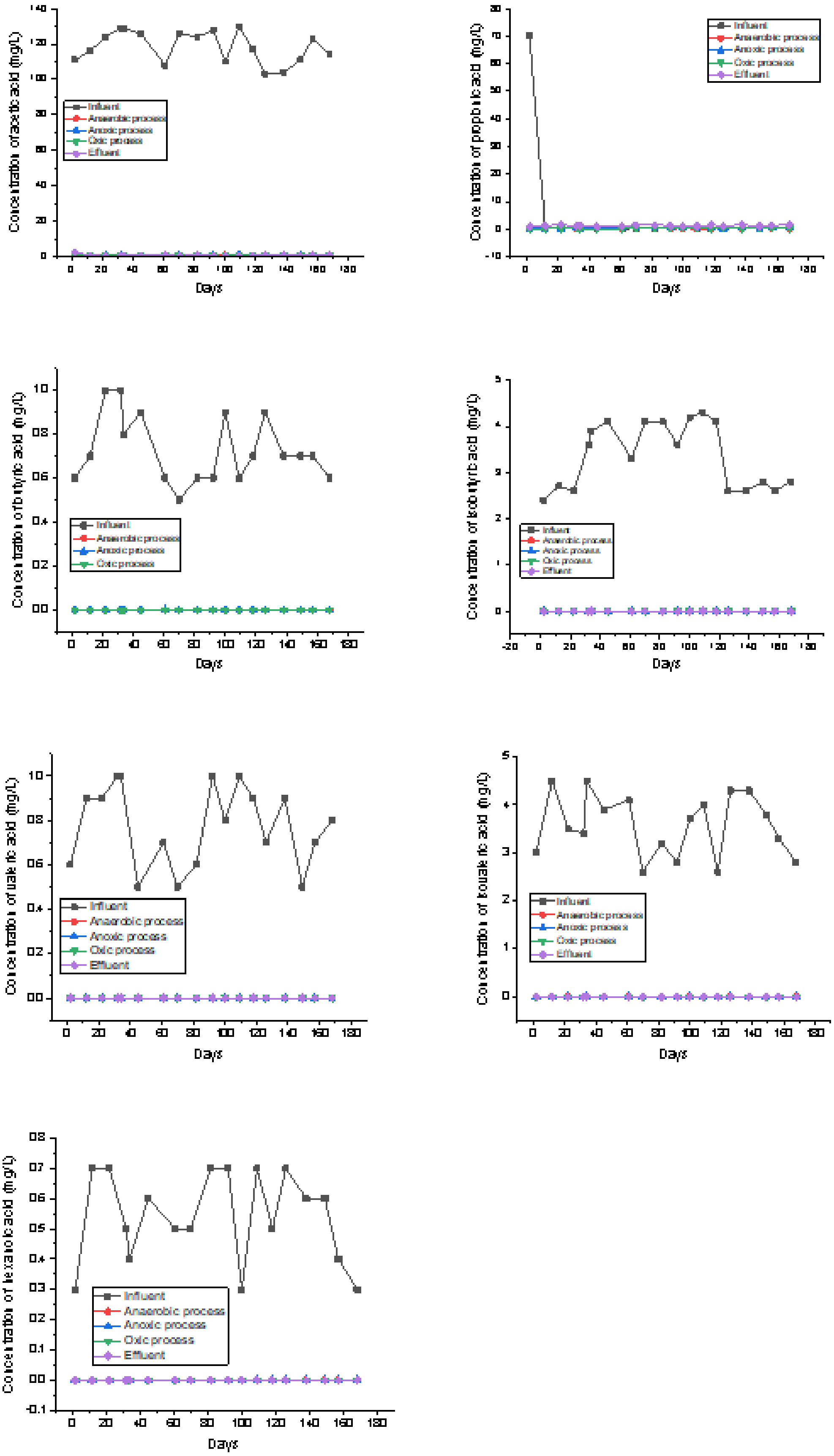

Figure 3-11. Volatile fatty acid monitoring during VFL unit operation

3.1.6 Summary

The experiment verifies that the VFL treatment process for high-concentration landfill leachate is economical and reasonable, and the treatment efficiency is high.

The high COD concentration can be effectively removed by the VFL device, and the removal rate can reach up to 86.5%. The effluent COD was stable in the whole stage, and the concentration fluctuation of the influent did not affect the effluent COD concentration.

- Although the overall NO_2^- concentration of the reactor is not high, the concentration of NO_2^- effluent is higher than that of the influent. The average NO_2^- concentration of the effluent was 18.7 mg/L. The average concentration of NO_3^- in the effluent is 22.6 mg/L, while the influent is only 5.0 mg/L. The average concentration of NH_4^+ in the effluent is 15.5 mg/L, which meets the requirements of China's national secondary emission standard, where the NH_4^+ concentration is lower than 25 mg/L, and the NH_4^+ removal rate is as high as 99.3%. This experiment has a good removal effect on total nitrogen (97.1%). The average total nitrogen concentration of the influent was 2635.7 mg/L, while the average total nitrogen concentration of the effluent was 77.4 mg/L.
- The degradation effect of total phosphorus is obvious. The total phosphorus concentration in each stage was relatively stable. The total phosphorus content in the influent water is high, at 10.3-15.7 mg/L. The total phosphorus content in the effluent is low, at 3.9-4.87 mg/L.
- The temperature shows a steady state first and then a decreasing trend. The highest temperature was as high as 28.6 °C, while the lowest temperature was 10.9 °C. The average pH of the influent is 7.8, the average pH of the outflow is 8.4, and the pH is slightly higher than that of the influent. The conductivity of the influent water is significantly higher than other stages. The VFL device degrades volatile fatty acids.

3.2 STUDY ON SLUDGE CHARACTERISTICS OF BEIJING LANDFILL LEACHATE TREATED BY VERTICAL FLOW LABYRINTH (VFL) DEVICE

The VFL treatment of landfill leachate is a complex biochemical reaction process with the joint action of multiple groups of microorganisms in the sludge. In the degradation process, microorganisms of various functional groups play their respective functions, cooperate and restrict each other, and finally form a dynamic and balanced micro-ecological environment [62]. The VFL device contains an anaerobic section, an anoxic section, and anaerobic section. For the characteristics of landfill leachate containing a high concentration of organic matter, it is suitable for advanced anaerobic biological treatment [63]. Because anaerobic treatment cannot remove other pollutants such as ammonia nitrogen and still contains high COD after treatment, it is generally connected to the aerobic process. The research in the previous chapter shows that the VFL device has a good removal effect on landfill leachate, which is benefited from the unique structure of the sludge and the interaction of a large number of microorganisms in it. Therefore, it is necessary to explore further the performance of VFL in degrading landfill leachate in terms of sludge characteristics.

3.2.1 MLSS variation of activated sludge in a vertical flow labyrinth (VFL) reactor

The operation of activated sludge requires reasonable adjustment of many control parameters, including the control of activated sludge concentration (MLSS), which is one of the most commonly used indicators in the daily operation of sewage systems and determines the sewage treatment capacity to a certain extent. The type and quantity of microorganisms in activated sludge will change correspondingly with the change in environmental conditions in the reactor. By analyzing the changes in activated sludge, the growth, reproduction, and metabolism of microorganisms in activated sludge can be preliminarily known. Understand the operating status of the reactor and the treatment effect. MLSS refers to the content of suspended solids in the mixed solution, and its unit is mg/L, which is used to measure the amount of activated sludge. The total amount of MLSS includes the following four aspects: active microorganisms, organic matter adsorbed on activated sludge that cannot be biodegraded, residues of microbial self-oxidation, and inorganic matter. MLSS can roughly obtain the microbial content of activated sludge in water [64]. In this experiment (Fig. 3-12), MLSS fluctuated wildly in August and September. The average value of MLSS in the anaerobic stage is 11350 mg/L, the average value in the anoxic stage is 12380 mg/L, and the aerobic stage is 11464 mg/L. Compared with the anaerobic stage, the MLSS of the anoxic stage increased by 9%; that is, the activity of microorganisms in the activated sludge was improved. In contrast, MLSS exceeding 15000 mg/L would hurt the reaction unit [65].

In this experiment, the slight increase of MLSS in the anoxic and aerobic sections may be that the microbial activity in the reactor gradually increased, and the microorganisms adapted to the internal environment of the reactor. Relevant researchers believe that MLSS is an essential factor affecting the effect of sewage treatment [66]. Plósz BG et al. thought that proper improvement of MLSS can affect the denitrification efficiency of sewage [67]. Brennan et al. explored the treatment effect of sewage treatment plants on landfill leachate. The experimental results show that MLSS is related to the pH of the reaction device. Under alkaline environmental conditions, MLSS will increase [68]. Tsilogeorgis et al. used a sequencing batch membrane bioreactor to treat landfill leachate. In their study, the initial MLSS was 7000 mg/L, and the MLSS rose to 15300 mg/L at the end of the reaction [69]. Insel et al. pointed out that when MLSS exceeds 13000 mg/L, the mass transfer of oxygen and nitrogen is limited, affecting the ammonia oxidation process [70].

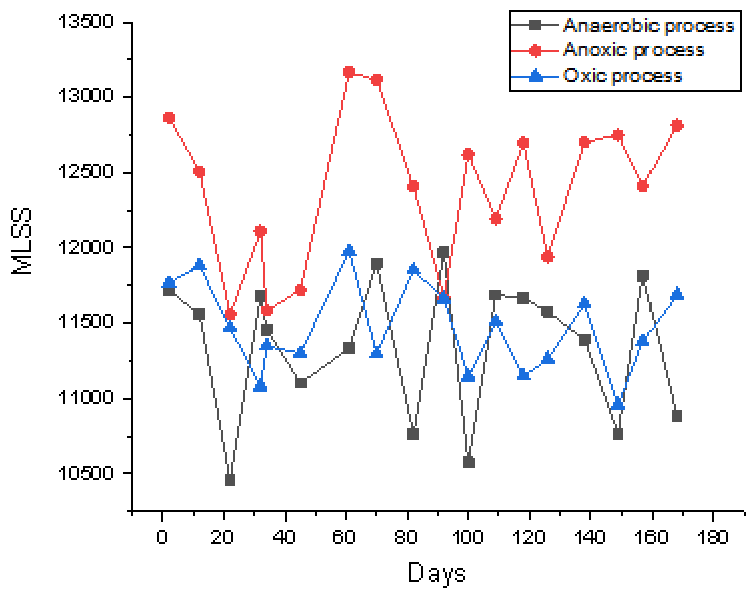

Figure 3-12. MLSS monitoring of activated sludge when VFL treats landfill leachate.

3.2.2 Vertical Flow Labyrinth (VFL) Reactor MLVSS Variation

Mixed liquid volatile suspended solids concentration (MLVSS), the concentration of organic solids in the mixed liquid activated sludge, refers to the mass of organic matter in the suspended solids contained in 1L of mixed liquid some researchers consider to be more accurate than MLSS.

It represents the number of microorganisms in activated sludge and indirectly reflects the survival status of microorganisms by monitoring the changes of MLVSS. Some researchers also pointed out that the indirect reduction of MLVSS mainly causes the reduction of MLSS. However, it is worth noting that MLVSS contains dead microbes, so it is not possible to draw exact conclusions. In this experiment, according to different sampling stages, the changes of MLVSS during the long-term treatment of landfill leachate by the VFL unit were monitored, as shown in Fig. 3-13. In this experiment, the MLVSS in the anoxic stage (8899.7 mg/L) > the MLVSS in the anaerobic stage (8458.8 mg/L) > the MLVSS in the aerobic stage (8205.6 mg/L). The MLVSS in the anoxic section is the largest and combined with the above MLSS; it is speculated that the environmental conditions in the anoxic section provide a more suitable attachment site for microorganisms, accelerate the metabolism of microorganisms, and thus promote the increase of MLVSS in the sludge mixture, and the growth rate is the fastest. Ranjan et al. used a sequencing batch reactor to investigate the effect of different concentrations of landfill leachate on its treatment effect. They found that regardless of the concentration of landfill leachate, MLVSS was always >2000 mg/L [71]. In addition, according to Duyar et al., MLVSS decreased due to high microbial growth rates and microbial release of extracellular secretions into the liquid phase [72].

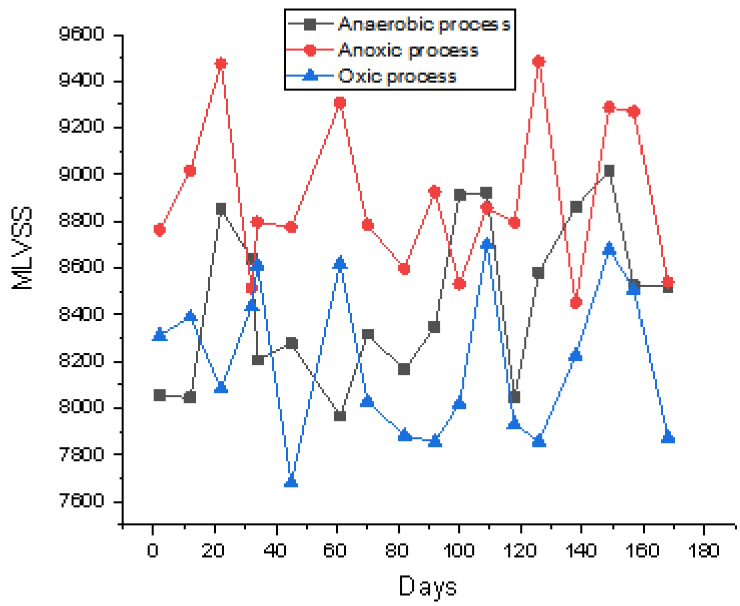

Figure 3-13. MLVSS monitoring of activated sludge when VFL treats landfill leachate.

3.2.3 Vertical Flow Labyrinth (VFL) Reactor MLVSS/MLSS Variation

MLVSS/MLSS represents the proportion of activated sludge in the total sludge, the proportion of organic solids in the sludge, and the active microbial biomass in the sludge [73]. The larger the MLVSS/MLSS, the higher the microbial biomass. In general, the ratio of MLVSS to MLSS is relatively fixed, such as in domestic wastewater, which is often around 0.75. The MLVSS/MLSS in this experiment was maintained at 0.72-0.75, indicating that the activated sludge in the system maintained a high activity (Fig. 3-14).

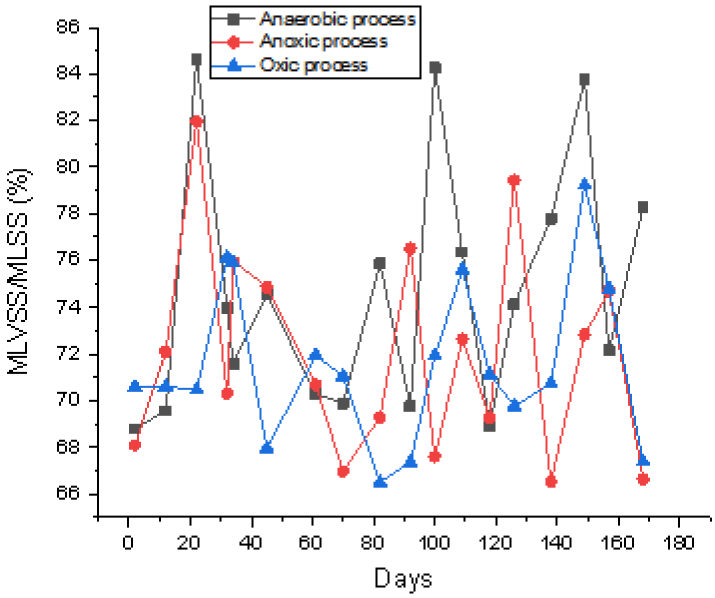

Figure 3-14. Changes in MLVSS/MLSS of activated sludge when VFL treats landfill leachate.

3.2.4 Sludge Morphology Change

Currently, several techniques have been proposed in the literature to describe the complex structure of sludge in terms of the material organization in the aggregates [74]. These techniques allow an understanding of the physical properties of the sludge (filament size and fractal dimension), particle size distribution (measured by photographic techniques such as free sedimentation, coulter counters, laser diffraction and malvern counters) and the effect of bio-flocculation on flow properties effects (rheological measurements and settling rates). Moreover, the recent development of image analysis technique has enabled a more complete understanding of the aggregates physical structure and morphology. Image technique has become a fundamental method with great applications within the Environmental Science [74].

In this study, activated sludge samples were taken at different stages of the VFL device, diluted with deionized water, and the granular sludge was carefully transferred to a glass slide. The morphology of the activated sludge was observed and photographed by an optical microscope. The morphology of activated sludge at room temperature at 400x magnification under an optical microscope is as follows (Fig. 3-15). The activated sludge in the anaerobic, anoxic, and aerobic stages all have irregular shapes, irregular edges, and brown color, and the color depth depends on the density of the sludge. Moreover, the activated sludge is flocculent, the internal density of the sludge is uneven, the center density is large, and the edges are sparse. The sludge in the anoxic stage was denser, while the sludge in the aerobic stage was relatively sparse.

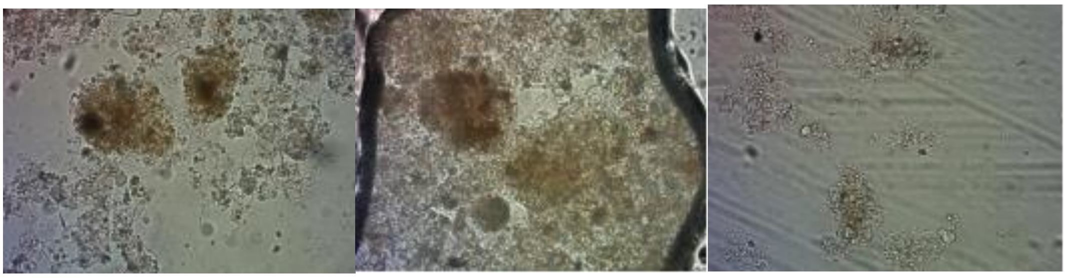

Figure 3-15. Morphological observation of microorganisms in activated sludge when VFL treats landfill leachate (400 times, in order: anaerobic stage, anoxic stage, aerobic stage).

3.2.5 Summary

Through long-term monitoring of activated sludge in the device during the VFL treatment of landfill leachate, the experimental results are as follows:

1) MLSS fluctuates significantly in August and September. The average value of MLSS in the anaerobic stage is 11350 mg/L, the average value in the anoxic stage is 12380 mg/L, and the aerobic stage is 11464 mg/L. Compared with the anaerobic stage, the MLSS of the anoxic stage increased by 9%, that is, the activity of microorganisms in the activated sludge was improved.

2) MLVSS in anoxic section (8899.7 mg/L)>anaerobic section MLVSS (8458.8 mg/L)>aerobic section MLVSS (8205.6 mg/L). The MLVSS in the anoxic section is the largest, and combined with the above MLSS, it is speculated that the environmental conditions in the anoxic section provide a more suitable attachment site for microorganisms, accelerate the metabolism of microorganisms, and thus promote the increase of MLVSS in the sludge mixture, and the growth rate is the fastest.

3) The MLVSS/MLSS in this experiment is maintained at 0.72-0.75, indicating that the activated sludge in the system supports a high activity.

4) The activated sludge in the anaerobic stage, anoxic stage, and aerobic stage all have irregular shapes, irregular edges, and brown color, and the color depth depends on the density of the sludge. Moreover, the activated sludge is flocculent, the internal density of the sludge is uneven, the center density is large, the edges are sparse, and there are still many flocs around the sludge that gather towards the center of the sludge. The sludge in the anoxic stage was denser, while the sludge in the aerobic stage was relatively sparse.

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Chapter 4

STUDY ON MICROBIAL COMMUNITY OF BEIJING LANDFILL LEACHATE TREATED BY VERTICAL FLOW LABYRINTH (VFL) DEVICE

STUDY ON MICROBIAL COMMUNITY OF BEIJING LANDFILL LEACHATE TREATED BY VERTICAL FLOW LABYRINTH (VFL) DEVICE

CHAPTER 4: STUDY ON MICROBIAL COMMUNITY OF BEIJING LANDFILL LEACHATE TREATED BY VERTICAL FLOW LABYRINTH (VFL) DEVICE

The rapid development of high-throughput sequencing technology provides a powerful means for analyzing the dynamic changes of sludge microbial community composition [1], identifying functional microbial flora and functional genes, and studying the interaction between microorganisms [2]. High-throughput sequencing technology can release fluorescent signals in the process of synthesis or ligation to generate new DNA [3], and has the characteristics of high sequencing throughput, short running time, and low cost. High-throughput sequencing has been applied to the study of microorganisms in different wastewater treatment systems. Metagenomic technology can study the genes and functions of microorganisms based on 16S rDNA sequencing [4-6]. The metagenomic sequencing analysis technology based on Illumina sequencing has developed rapidly, and the cost of sequencing analysis has gradually decreased [7]. It has shown significant advantages in the in-depth study of microbial communities and has been widely used in drinking water, laboratory simulation reactors, urban sewage treatment plants, etc. Analysis of microbial communities in the environment [8, 9]. Arguably, metagenomic sequencing analysis has proven to be a powerful tool for unraveling the “dark matter” in engineered ecosystems of higher diversity [10, 11]. For example, Zhang et al. used metagenomic sequencing technology to study the microbial community structure of four sewage treatment plants. They found that the microbial community of activated sludge treated domestic sewage had higher diversity than industrial sewage [12]. Fang et al. explored the dominant bacterial genera of activated sludge from pesticide wastewater treatment plants. They found that bacterial genera were different from those of activated sludge from industrial wastewater and municipal wastewater [13]. Joshi et al. conducted a study on the biological treatment system of coking wastewater, and the results showed that *Burkholderiales*, *Actinomycetales*, *Rhizobiales*, *Pseudomonadales* and *Hydrogenophiliales* are important functional microbial groups. Aromatic dioxygenase, digestive enzyme and thiocyanate hydrolase genes are nitrogen- and sulfur-containing—important functional genes for the biotransformation of pollutants [14]. The microbial community structure and its biological mechanism have an essential influence on the function and effect of the reactor. To clarify the differences in the microbial community structure and metabolic pathways in different stages of landfill leachate treated by the VFL unit for a long time, this experiment used metagenomic sequencing technology to explore the microbial distribution law and analyzed the community structure of the dominant bacteria in the VFL reactor. And feature analysis.

4.1 Rarefaction curve

The Rarefaction curve is completed by using the number of individuals and the number of species in the sample [15, 16]. Generally, the abscissa is the amount of high-throughput sequencing data, and the ordinate is the number of species at the corresponding level. The rationality of high-throughput sequencing and the feasibility of data analysis can be clarified by analyzing the rarefaction curve [17, 18]. It can also be used to compare the species richness of samples with different sequencing numbers, and it can also be used to indicate whether the sampling size of the sample is reasonable. When the curve tends to be flat, the sampling depth has covered all species in the sample, and more data contributes less to discovering new OTUs [19]. On the contrary, the species diversity in the sample is high, and there are still undetected species, continuous sampling may also generate more and new OTUs. Figure 1 shows the dilution curves inside the VFL device in 3 time periods (2021.7.9, 2021.10.13, 2021.12.12). It can be seen that the sample dilution curve tends to be flat, indicating that the amount of sequencing data is large enough, and the depth of sequencing is sufficient to represent most microbial species in the sample, which can reflect the vast majority of microbial diversity information in the model (Fig. 4-1).

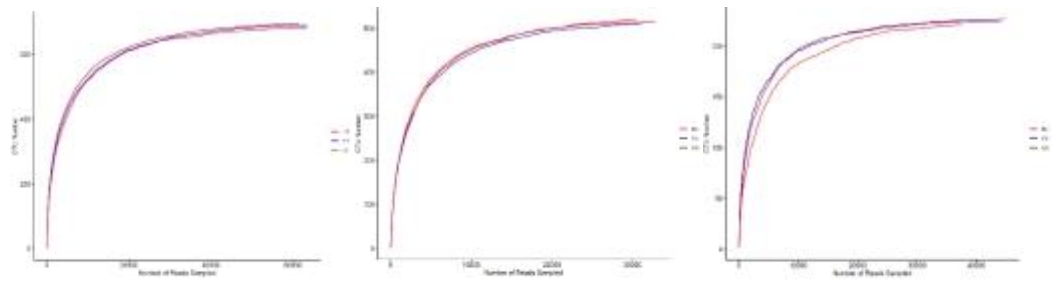


Figure 4-1. Dilution curves inside the VFL unit in 3 time periods (2021.7.9, 2021.10.13, 2021.12.12), Y: anaerobic, Q: anoxic, H: aerobic.

4.2 Alpha diversity analysis of overall microbial community structure

Alpha biodiversity refers to the biodiversity of each sample [20]. There are many indicators.

Here, five indicators are mainly calculated, including observed species index (OTUs), Chao1 index, Shannon index, Simpson index, and PD whole tree [21]. Chao index and Ace index are used to estimating the number of OUT in the sample; the larger the value, the more abundant the species in the model [22, 23]. The Simpson index and Shannon index reflect the diversity and evenness of species in the sample [24]. The larger the Simpson index, the lower the biodiversity of the biological community, and the higher the value of the Shannon index, the higher the biodiversity of the biological community (Table 4-1).

The OTUs of the three time periods are different, and the order from large to small is 2021.7.9, 2021.10.13, 2021.12.12. However, the OTUs of the three samples with other distributions in the VFL device were not significantly different. The OTUs of the samples collected on July 9, 2021, ranged from 683 to 695, the OTUs of the samples collected on October 13, 2021, ranged from 512 to 521, and the samples collected on December 12, 2021. The collected sample OTUs ranged from 221-227.

The Ace index uses rare species to estimate the index of species diversity [25]. The higher the value, the richer the species of the community. The Ace index is used to estimate the number of OUTs contained in a colony [26]. The Ace index of the three-time periods was consistent with the change of OTUs, and the index changed obviously due to the temperature change. The order from large to small is 2021.7.9, 2021.10.13, 2021.12.12. The difference in OTUs of the three samples with different distributions in the VFL device is relatively insignificant, and the Ace range of the samples collected on 2021.7.9 is between 691.2236-705.6699, the Ace range of the samples collected on 2021.10.13 is 521.3161-530.6069, and the Ace range of the pieces collected on 2021.12.12 is 226.3636-229.559.

Chao1 index is an index used to reflect species richness [27]. It has nothing to do with abundance and evenness, but it is sensitive to rare species [28]. The Chao1 index of 3 samples with different distributions in 3 time periods is different, and the changes are obvious. The sample distribution range of 2021.7.9 is 689.6327-705.3333. Generally speaking, the Chao1 index from large to small order is Q1, Y1, H1 The sample distribution range of 2021.10.13 is 521.2195-530.6923. Generally speaking, the Chao index is H5, Q5, and Y5 in descending order. The sample range of 2021.12.12 is 226.3636-228.6471. Generally speaking, the Chao index in Q8, H8, Y8 in descending order. It can be seen that the impact of temperature on biodiversity is significant, and anaerobic, anoxic, and aerobic inside the VFL device also have a particular impact on biodiversity. Still, the effect is not so much compared to temperature obvious.

The Shannon index is used to describe the disorder and uncertainty of the individual occurrence of a species [29]. The higher the uncertainty, the higher the diversity. Two factors are included in the Shannon index: the number of species, that is, abundance; and the average or even distribution of individuals in the species. A large number of species increases diversity, and similarly, an increase in the uniformity of individual distribution among species also increases diversity. The Shannon exponents of the three-time periods vary significantly due to temperature changes. The order from big to small is 2021.7.9, 2021.10.13, 2021.12.12. That is, biodiversity from high to low is 2021.7.9, 2021.10.13, 2021.12.12, the temperature decreases, and the biodiversity decreases. The difference in the Shannon index of the three samples with different distributions in the VFL device is relatively insignificant; the Ace range of the samples collected on 2021.7.9 is between 4.6185-4.7202, the Ace range of the pieces collected on 2021.10.13 is between 4.0653-4.2535, and the sample collected on 2021.12. The Ace of 12 collected samples ranged from 2.0622 to 2.6994.

Rare species play a more minor role in the Simpson Diversity Index, while common species play a more significant role. In this experiment, the samples with the most extensive Simpson diversity index were collected on December 12, 2021. In addition, for the three samples with different distributions in the three-time periods, the coverage distribution range is 0.9991-0.9999, which is consistent with the dilution curve in the previous section, which also indicates that the sequencing data is sufficient to reflect the information of most bacterial communities in the samples. In general, the higher the microbial abundance, the more complex the community composition structure, the higher the microbial community abundance, the faster the material metabolism, and the stronger the functional stability of the microbial ecology [30]. We analyzed that the decrease in temperature is not conducive to the reproduction of microorganisms involved in the degradation of landfill leachate, leading to a decrease in microbial diversity in the VFL unit.

Table 4-1. Bacterial community diversity index at various stages of VFL

Time	Sample	OTUs	Ace	Chao1	Shannon	Simpson	Coverage
2021.7.9	Y1	689	699.8169	701.7179	4.7202	0.0251	0.9995
	Q1	695	705.6699	705.3333	4.7091	0.0272	0.9995

	H1	683	691.2236	689.6327	4.6185	0.0292	0.9996
2021.10.13	Y5	512	521.3161	521.2195	4.1299	0.0646	0.9991
	Q5	516	522.3230	522.1765	4.0653	0.0751	0.9994
	H5	521	530.6069	530.6923	4.2535	0.0565	0.9991
2021.12.12	Y8	225	226.614	226.3636	2.6994	0.1809	0.9999
	Q8	227	229.559	228.6471	2.5450	0.2038	0.9998
	H8	221	228.225	227.1818	2.0622	0.2811	0.9995

4.3 Analysis of differences in anaerobic-anoxic-aerobic microbial community structure

Each sample was analyzed at the phylum level to explore the composition of microbial communities in the system at different times. The results are shown in Fig. 4-2. Among the samples sampled on 2021.7.9, the Q1 community contained, Proteobacteria (41.86%), Actinobacteria (2.17%), unclassified_Bacteria (14.48%), Deinococcus-Thermus (2.69%), Chloroflexi (11.41%), Ignavibacteriae (3.5%), Planctomycete (10.01%), Bacteroidetes (9.87%), Other (4.02%).

Y1 community contains, Proteobacteria (41.02%), Actinobacteria (2.48%), unclassified_Bacteria (16.15%), Deinococcus-Thermus (3.17%), Chloroflexi (10.86%), Ignavibacteriae (3.47%), Bacteroidetes (10.53%), Planctomycetes (8.67%), Other (3.66%). H1 community contains, Proteobacteria (41.52%), Actinobacteria (1.97%), unclassified_Bacteria (15.16%), Deinococcus-Thermus (2.2%), Planctomycetes (11.46%), Bacteroidetes (10.21%), Ignavibacteriae (4.51%), Chloroflexi (9.42%), Other (3.55%).

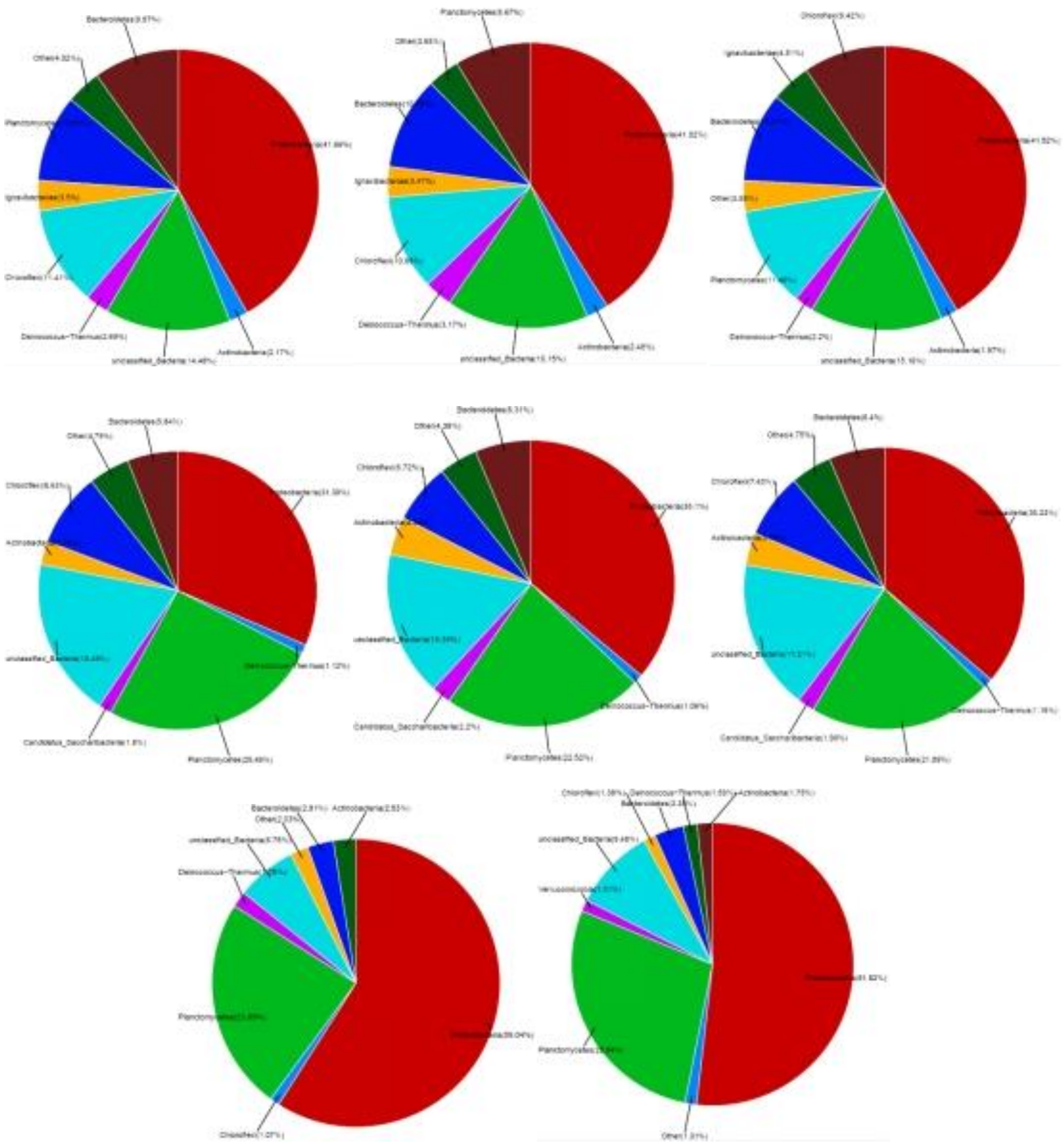
Among the samples sampled on 2021.10.13，Q5 community contains，Proteobacteria (31.39%), Deinococcus-Thermus (1.12%), Planctomycetes (25.49%), Candidatus_Saccharibacteria (1.5%), unclassified_Bacteria (18.49%), Actinobacteria (2.75%), Chloroflexi (8.63%), Bacteroidetes (5.84%), Other (4.79%). Y5 community contains, Proteobacteria (36.1%), Deinococcus-Thermus (1.06%), Planctomycetes (22.52%), Candidatus_Saccharibacteria (2.2%), unclassified_Bacteria (16.39%), Actinobacteria (4.33%), Chloroflexi (6.72%), Bacteroidetes (6.31%), Other (4.36%).

H5 community contains, Proteobacteria (36.23%), Deinococcus-Thermus (1.16%), Planctomycetes (21.09%), Candidatus_Saccharibacteria (1.98%), unclassified_Bacteria (17.21%), Actinobacteria (3.75%), Chloroflexi (7.43%), Bacteroidetes (6.4%), Other (4.75%). Q8 community contains, Proteobacteria (59.04%), Chloroflexi (1.07%), Planctomycetes (23.89%), Deinococcus-Thermus (1.78%), unclassified_Bacteria (6.75%), Bacteroidetes (2.91%), Actinobacteria (2.53%), Other (2.03%).

Y8 community contains, Proteobacteria (51.82%), Planctomycetes (28.04%), Verrucomicrobia (1.31%), unclassified_Bacteria (9.45%), Chloroflexi (1.38%), Deinococcus-Thermus (1.59%), Bacteroidetes (3.35%), Actinobacteria (1.75%), Other (1.31%). H8 community contains， Proteobacteria (50.78%)，Deinococcus-Thermus (1.05%)，Planctomycetes (37.71%)，Bacteroidetes (3.05%)， unclassified_Bacteria (5.08%)， Other (2.33%).

Anammox bacteria belong to Planctomycetes [31, 32]. Denitrifying bacteria generally belong to Proteobacteria, Firmicutes and Bacteroidetes, whereas dissimilatory nitrate-reducing bacteria to ammonium generally exist in Proteobacteria and Bacteroidetes [33, 34]. In addition, from the above results, it can be concluded that Proteobacteria is the dominant flora, which is similar to previous reports [35, 36]. In the sewage treatment systems, Proteobacteria flora is a kind of dominant flora, most of which are facultative or obligate anaerobic Gram-negative. Most denitrifying microorganisms and denitrifying bacteria belong Proteobacteria [37]. Proteobacteria can use glucose, propionic acid, butyric acid, and acetic acid as substrates and are acetate-consuming bacteria [38]. Several studies have demonstrated that Proteobacteria can degrade various organic pollutants, remove nitrogen and phosphorus, and reduce the biological toxicity caused by contaminants [39-41]. Bacteroidetes are involved in the degradation of polymers and complex organic matter. They can break down dead cells containing polysaccharides and proteins into simple organic molecules (such as ethanol and lactic acid) that can be metabolized by other species [42, 43]. In addition, Bacteroidetes have the strong metabolic capacity for complex organic matter, proteins and lipids, etc. They can decompose complex macromolecular substances into simple compounds, which play an important role in ecosystems [44]. Firmicutes are a group of gram-positive bacteria that can produce spores and resist extreme environments. The bacterial wall has a high peptidoglycan content, and its classified Bacillus (Clostridia) may be related to refractory organic matter. Related. Chloroflexi are mostly filamentous bacteria, and they can exist in the form of floc skeleton inside the sludge bacteria micelle flocs, which can promote sludge flocculation [45] and can synthesize exopolysaccharides; it plays an important role in the formation of sludge agglomerates, and can enhance the structure of biofilms through a filamentous network [46-48]. It also can degrade macromolecular organic matter and has an excellent biological phosphorus removal effect [49].

Comparatively, Xie et al. used an anaerobic dynamic membrane bioreactor to treat landfill leachate, in their study, most of the major phyla were Firmicutes, Bacteroidetes, TM6, Chloroflexi, Actinobacteria and Proteobacteria, which in total accounted for 63.1% (91 d), 91.8% (106 d) and 93.0% (141 d) of the entire sequence reads, respectively [50]. This has some similarities with our experimental results. Besides, Ma et al. reported that the majority of these phyla were ubiquitous in lab-scale and pilot-scale anaerobic bioreactors [51].



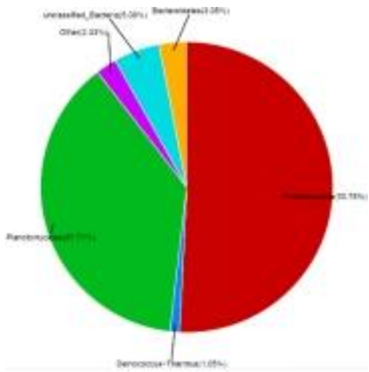


Figure 4-2. VFL device community composition based on phylum classification level (Q, Y, H from left to right; 2021.7.9, 2021.10.13, 2021.12.12 from top to bottom)

Each sample was analyzed at the family level to explore the composition of microbial communities in the system at different times. The results are shown in Fig. 4-3. 2021.7.9 sampled in a sample comprising community Rhodocyclaceae, Anaerolineaceae, Ignavibacteriaceae, Nitrosomonadaceae, Trueperaceae, Saprospiraceae, Xanthomonadacease, Rhodobacteraceae, Methylophilaceae, Planctomycetaceae, unclassified_Bacteria, unclassified_Planctomycetes, unclassified_Gammaproteobacteria, unclassified_Bacteroidetes, unclassified_Proteobacteria, unclassified_Actinobacteria, unclassified_Sphingobacteriales, unclassified_Chloroflexi, unclassified_Burkholderiales, unclassified_Chromatiales, unclassified_Betaproteobacteria.

In the samples sampled on 2021.10.13, the community contains Rhodocyclaceae, Anaeroloneaceae, Nitrosomonadaceae, Saprospiraceae, Xanthomonadaceae, norank_Candidatus_Saccharibacteria, Rhodospirillaceae, Trueperaceae, unclassified_Planctomycetes, unclassified_Bacteria, unclassified_Gammaproteobacteria, unclassified_Acidimicrobidae, unclassified_Burkholderiales, unclassified_Betaproteobacteria, unclassified_Bacteroidetes, unclassified_Chloroflexi, unclassified_Micrococcineae. In the samples sampled on 2021.12.12, the community contains Rhodocyclaceae, Rhodobacteraceae, Saprospiraceae, Nitrosomonadaceae, Trueperaceae, Rhodospirillaceae, norank_Spartobacteria, Anaerolineaceae, unclassified_Planctomycetes, unclassified_Gammaproteobacteria, unclassified_Bacteria, unclassified_Burkholeroales, unclassified_Micrococcineae.

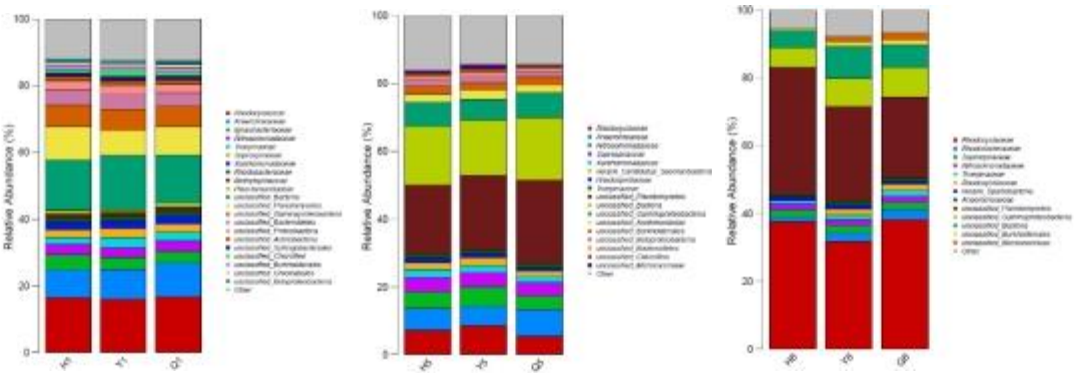


Figure 4-3. VFL device community composition based on family taxonomy level (from left to right: 2021.7.9, 2021.10.13, 2021.12.12)

Each sample was analyzed at the genus level to explore the composition of microbial communities in the system at different times; the results are shown in Fig. 4-4. In the samples of 2021.7.9，H1 mainly contains *Thauera* (15.795%)、*Ignavibacterium* (3.887%)、*Nitrosomonas* (3.035%)、*Truepera* (2.197%)、*Pseudofulvimonas* (0.987%)、*Lewinella* (0.891%)；Y1 mainly contains *Thauera* (15.339%)、*Ignavibacterium* (3.012%)、*Nitrosomonas* (2.925%)、*Truepera* (3.171%)、*Pseudofulvimonas* (1.026%)、*Lewinella* (1.026%)；H1 mainly contains *Thauera* (15.864%)、*Ignavibacterium* (2.973%)、*Nitrosomonas* (3.062%)、*Truepera* (2.685%)、*Pseudofulvimonas* (1.071%)、*Lewinella* (0.974%). In addition， in the samples of 2021.10.13，H5 mainly contains *Thauera* (6.066%), 、*Nitrosomonas* (4.976%), 、*Pseudofulvimonas* (1.861%)、*Truepera* (1.162%); Y5 mainly contains *Thauera* (7.433%)、*Nitrosomonas* (5.838%)、*Pseudofulvimonas* (1.666%)、*Truepera* (1.062%)； Q5 mainly contains *Thauera* (4.581%)、*Nitrosomonas* (4.031%)、*Pseudofulvimonas* (1.405%)、*Truepera* (1.116%). In the samples of 2021.12.12， H8 mainly contains *Thauera* (36.918%), 、*Nitrosomonas* (1.742%)、*Truepera* (1.045%); Y8 mainly contains *Thauera* (30.787%), 、*Nitrosomonas* (1.638%)、*Truepera* (1.593%); Q8 mainly contains *Thauera* (37.220%)、*Nitrosomonas* (1.776%)、*Tr*uepera (1.778%).

Thauera was the predominant genus at the genus level regardless of whether samples were collected at different times or from other distribution areas within the VFL unit, as in previous studies. Thauera is a common genus of denitrifying bacteria belonging to the *β-Proteobacteria* in the Proteobacteria phylum. *β-Proteobacteria* are generally considered to be the prominent members of the activated sludge community, a finding that has also been reported in other started sludge microbial studies [35]. In addition, the main genus of ammonia-oxidizing bacteria in the system is

Nitrosomonas, and *Nitrosomonas* communis and *Nitrosomonas* urea are common species in this genus, which are often detected in existing sewage plants [52]. Studies have also shown that in the anammox system for treating landfill leachate, the dominant genus of anammox bacteria is *Candidatus Kuenenia* [53], which may be due to the strong affinity of *Candidatus Kuenenia* to the substrate, which can be a better adaptation to landfill leachate [54]. Overall, the variation of ammonia-oxidizing bacteria in the installation may be due to the succession that occurs due to seasonal changes, and seasonal temperature changes have been reported to be a critical factor in the line of nitrifying bacteria communities [8].

Venn diagrams can more intuitively count each sample's common and unique population categories to understand their compositional similarity and overlap [55, 56]. The 2021.7.9 samples reflected 198 species contained in the anaerobic, anoxic, and aerobic sections; the 2021.10.13 samples reflected 160 species included in the anaerobic, anoxic, and aerobic cells; 2021.12. The 12 samples reflected 83 species in the anaerobic, anoxic, and aerobic segments. In the 2021.12.12 sample, the microbial diversity of the anaerobic and aerobic sections is included in the anoxic area.

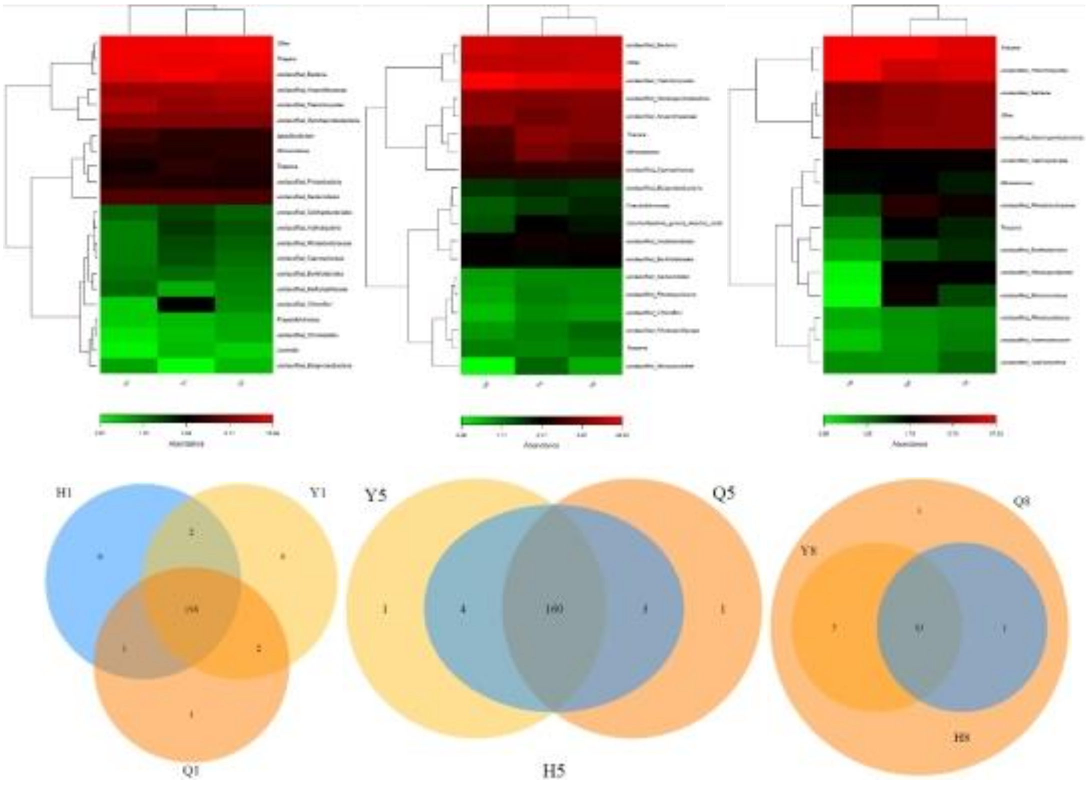


Figure 4-4. Heatmap of community composition of VFL devices based on the genus taxonomy level in the upper layer (from left to right: 2021.7.9, 2021.10.13, 2021.12.12) and the lower layer based on the intersection of VFL device communities at the genus taxonomy level.

4.4 Anaerobic-anoxic-aerobic microbial community-related gene functions in VFL devices

It can be seen from Fig. 4-5 that 47 gene functions can be predicted from bacterial sequences in the VFL device. In addition to having a solid basic function of maintaining cell life activities, nitrification and denitrification, photoautotrophy and photosynthesis, and carbohydrate transport and metabolism have relatively high potential in terms of functional proportions.

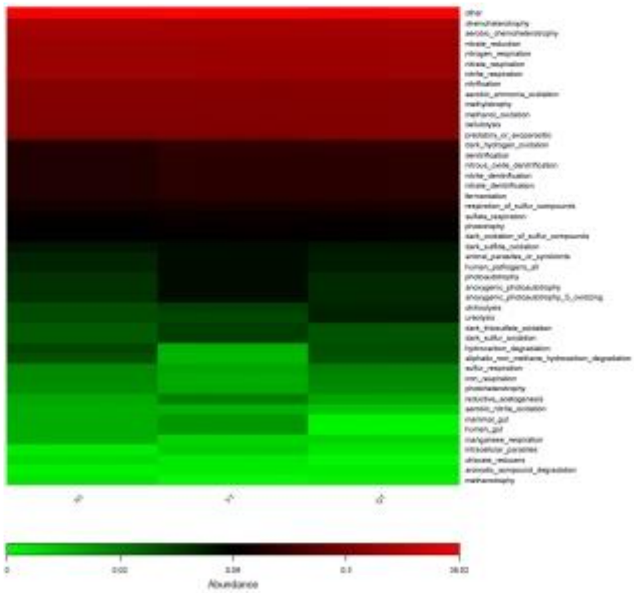


Figure 4-5. Detection of microbial community-related functional genes in the VFL device, 2021.7.9.

Compared with the samples collected in 2021.7.9, there are 47 genes predicted in 2021.10.13. In addition to the above functions, that is, in addition to the solid basic functions of maintaining cell life activities, nitrification and denitrification, and light energy, The difference between nutrition and light energy and the potential of carbohydrate transport and metabolism functions are also relatively high in function ratio. The fermentation effect is also more prominent (Fig. 4-6).

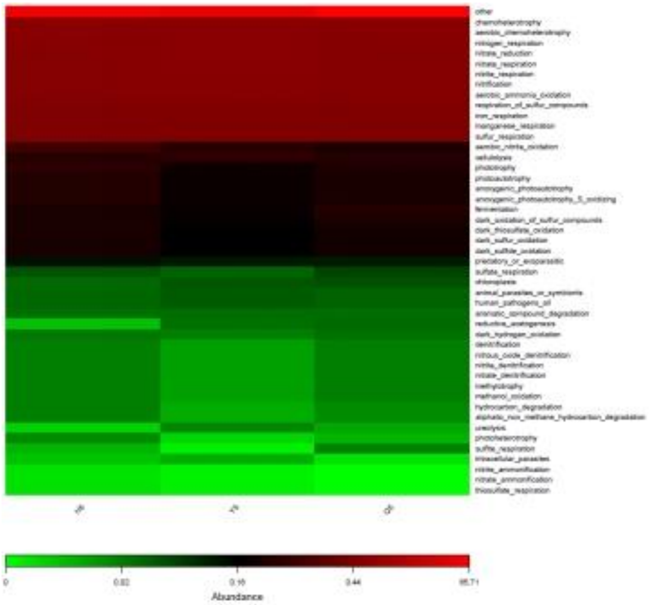


Figure 4-6. Detection of microbial community-related functional genes in the VFL device, 2021.10.13

Different from the previous two times (samples collected on 2021.7.9 and 2021.10.13), there are 34 genes predicted in the samples collected on 2021.12.12, the microbial function has decreased, the denitrification has reduced, but the methyl nutrition is abnormally rich (Fig. 4-7).

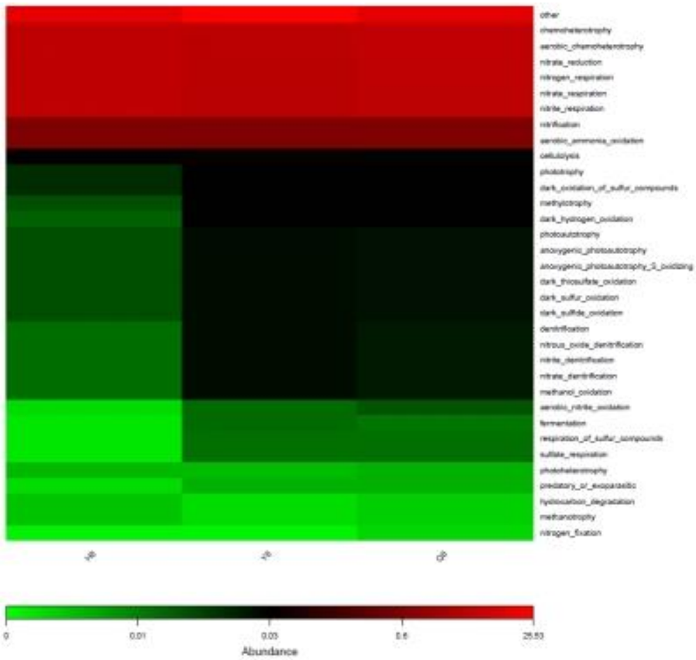


Figure 4-7. Detection of microbial community-related functional genes in the VFL device, 2021.12.12.

4.5 Summary

In this chapter, metagenomic sequencing technology was used to analyze the microbial community structure of the sludge samples in the anaerobic, anoxic, and aerobic sections of the long-running VFL unit for the treatment of landfill leachate. The conclusions are as follows:

1) The sample dilution curve tends to be flat, indicating that the amount of sequencing data is large enough. The sequencing depth is sufficient to represent most microbial species in the sample, which can reflect the vast majority of microbial diversity information in the model.

2) In general, the higher the microbial abundance, the more complex the community composition structure, the higher the microbial community abundance, the faster the material metabolism, and the stronger the functional stability of the microbial ecology. The decrease of seasonal temperature, it is not conducive to the reproduction of microorganisms involved in the degradation of landfill leachate, which leads to a relative reduction in the microbial diversity in the VFL device.

3) Proteobacteria, Actinobacteria, unclassified_Bacteria, Deinococcus-Thermus, Chloroflexi, Ignavibacteriae, Planctomycete at the phylum level, Bacteroidetes were the dominant flora. At the genus level, *Thauera*, *Ignavibacterium*, *Nitrosomonas*, *Truepera*, *Pseudofulvimonas*, *Lewinella* were the dominant communities.

4) The bacterial sequences in the VFL device can predict 47 gene functions. In addition to the solid basic functions of maintaining cell life activities, nitrification and denitrification, photoautotrophy and photosynthesis, and carbohydrate transport and denitrification. The potential for metabolic function is also relatively high in the proportion of parts. However, with the decrease in seasonal temperature, the microbial process decreased and denitrification decreased, but methyl nutrient was abnormally abundant.

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Chapter 5

RESEARCH ON THE TREATMENT OF INDUSTRIAL DYEING AND PRINTING WASTEWATER

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5.1 RESEARCH ON THE TREATMENT OF INDUSTRIAL DYEING AND PRINTING WASTEWATER BY ANAEROBIC REACTOR

Nowadays, with the rapidly increasing population worldwide, it gives the effect, which directly or indirectly affects the environment with widespread urbanization, and mushrooming of industries means escalated water utilization and pollution resulting from the never-ending waste disposal problem [1]. In addition to the existing problem is the continuous reform and new legislative restrictions that are being imposed by individual governments worldwide in view of the safety and health concerns of the citizens to make sure the treated effluent follows the strict conditions with the certain standard before being exposed to water streams [2-5]. Mostly, advanced treatment for high strength wastewater is needed due to the diverse nature of the water streams coming from the various domestic and industrial sources such as the production of drugs and pharmaceutics, pesticides, food processing, fermentation, nuclear processing, poultry, dairy, sugar and molasses, pulp and paper mill, rubber and palm oil mill effluent (POME) which contain a high load of chemicals, heavy metals, organic and inorganic material which are varied in physical and biological [6-11].

Hence, the treatment methods are varied to remove heavy metals, organic/inorganic pollutants, and chemicals based on advanced primary biological/chemical treatment for a different type of industrial wastewater either treated in or on-site installation [12]. Therefore, the significance of industrial wastewater treatment becomes crucial and further research in low-cost green technology development must be implemented to clean the wastewaters and achieve a benchmark to make them fully portable [13]. If not, they at least should be used for agricultural and non-potable purposes and protects the environment from the contaminants of wastewaters [14-16].

As a traditional pillar industry in China, the textile industry produces 1.84 billion tons of wastewater every year, causing serious environmental pollution [17]. Textile printing and dyeing wastewater has the characteristics of high pH, high turbidity, poor biodegradability, complex composition, high chroma, and large discharge, and is considered to be one of the most difficult industrial wastewaters to treat [18, 19].

Currently, physicochemical technologies are commonly used for the treatment of industrial wastewater, because of their simple operation and high removal efficiency [20]. However, these methods are usually energy-intensive and expensive [21-23]. Alternatively, biological processes with limited energy consumption, low cost, and high efficiency are considered promising technologies [24]. For example, the aerobic activated sludge technology can be used for the treatment of industrial wastewater, although it still faces certain intractable problems such as sludge expansion, solid sludge treatment, and foam formation [25]. In contrast, anaerobic processes can avoid these existing limitations of their aerobic counterparts and have been proven effective for treating high-concentration textile wastewater with improved performance [26]. For instance, the up-flow anaerobic sludge bed (UASB) reactor is one of the most widely applied anaerobic reactors for textile wastewater treatment because of its public UASB patent [27]. However, this technology suffers from other issues such as unsafe operation, sludge wash-out, and granular sludge disintegration [28]. To address these problems, several advanced third-generation anaerobic reactors such as internal circulation (IC) reactors and expanded granular sludge bed (EGSB) reactors have been developed and successfully applied in several industries (e.g., beer production, food processing, and papermaking). However, to the best of our knowledge, only very few reports are available on the application of effective bench-scale anaerobic reactors to practical textile wastewater treatment [29] and in most cases the target pollutants are synthetic dyeing compounds.

In this chapter, an anaerobic reactor system was developed, a schematic representation of the reactor is present in Fig. 5-1, and used for the treatment of industrial printing and dyeing wastewater for the first time. The treatment performance is examined systematically and the key operational parameters are identified.

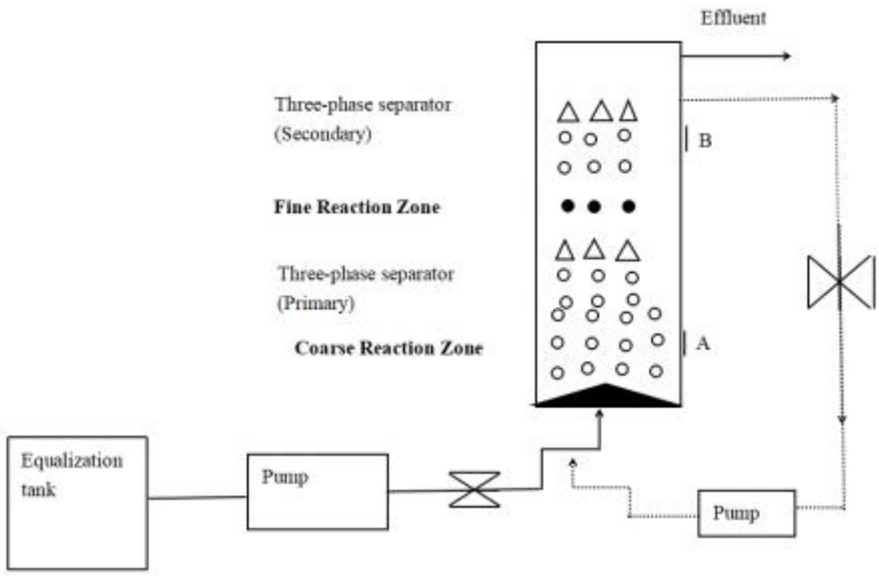

Figure 5-1. A schematic representation of the reactor system.

5.1.1 Effect of pH on COD removal

pH is the most critical factor in the anaerobic process [30]. For Fig. 5-2 and 5-3, COD removal increased when pH increased. It is generally believed that the optimum pH range is 6.8 to 7.2. At <6.5 or >8.2, methanogens are severely inhibited, leading to the deterioration of the entire anaerobic reaction process.

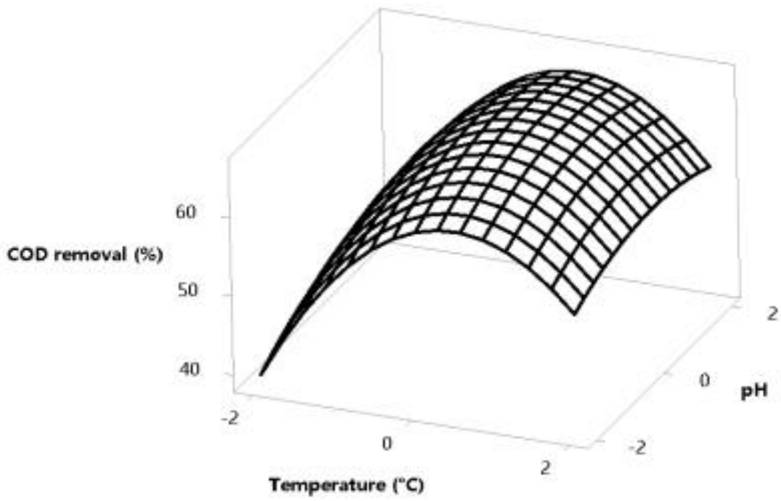

Figure 5-2. Surface Plot of COD removal (%) vs. pH, Temperature (°C).

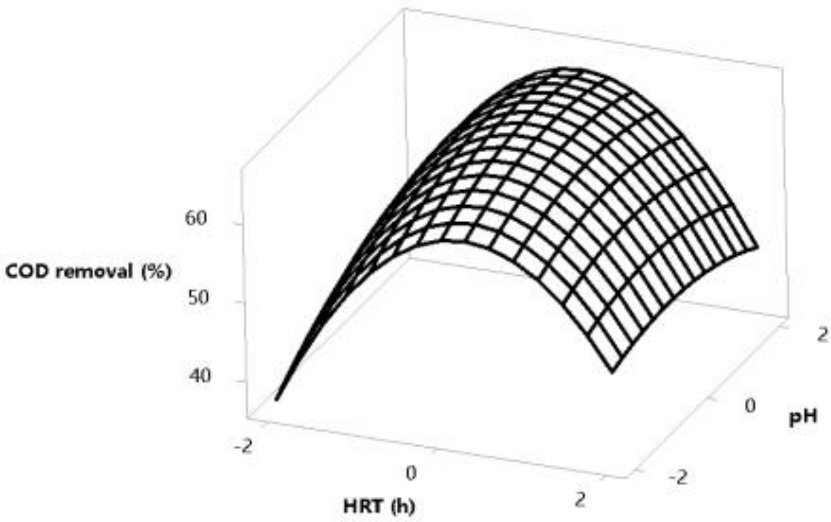

Figure 5-3. Surface Plot of COD removal (%) vs. pH, HRT (h).

5.1.2 Effect of temperature on COD removal

The suitable temperature for anaerobic sometimes varies to some extent due to other process conditions [31]. For example, a higher sludge concentration in the reactor, that is, a higher concentration of microbial enzymes makes the influence of temperature less likely to be revealed. In a specific temperature range, the temperature increases, and the organic matter removal rate increases. In general, the temperature rises by 10°C for all other process conditions, and the reaction rate is increased by about 2 to 4 times. For Fig. 5-4, it can be known that COD removal increased when the temperature increased.

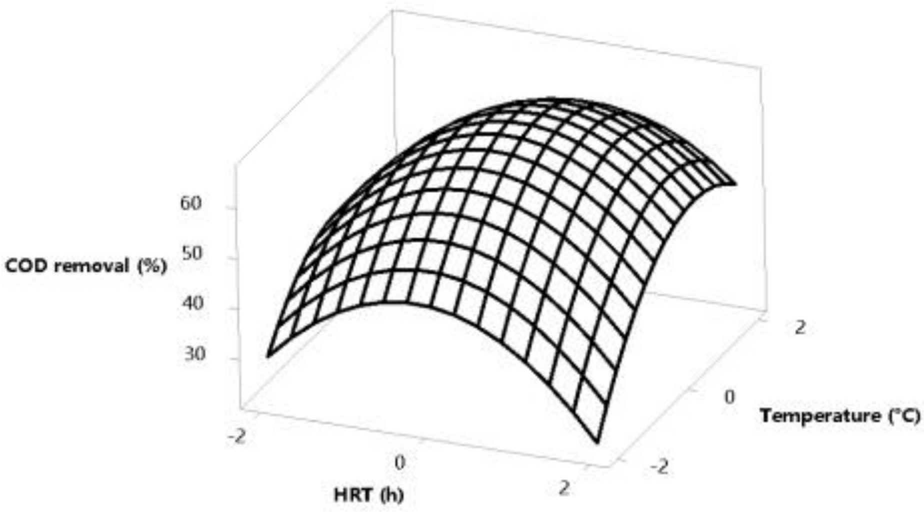

Figure 5-4. Surface Plot of COD removal (%) vs. Temperature (°C), HRT (h).

5.1.3 Effect of reflux ratio on COD removal

The reflux ratio is an essential parameter for determining the performance of an anaerobic reactor [32]. This study observed the effects of the reactor reflux ratio on COD removal under an optimized HRT of 8.5 h; the corresponding results are summarized in Fig. 5-5 and 5-6. During the experiment, the influent COD varied significantly from 1400 to 4200 mg/L because the industrial dyeing wastewater employed is comprehensive wastewater including desizing wastewater, dyeing wastewater, and printing wastewater. Its main components are dyes, slurry (e.g., starch, PVA, CMC), terephthalic acid, glycol, fiber and surfactants. Moreover, the water characteristics also depend on the changes in actual experimental conditions (e.g., the order quantities). Meanwhile, the effluent COD concentration changed from 800 to 2000 mg/L accordingly. As shown in Fig. 6-5 and 6-6, the COD removal increased gradually with the reflux ratio from 0 to 4. In particular, the average COD removal efficiency was 34.2%, 42.9%, and 55.8%, respectively, at the reflux ratio of 0, 2, and 4. The maximum COD removal efficiency of 64.99% was obtained at the reflux ratio of 4. However, further increasing the reflux ratio to 5 adversely affected the COD removal with a limited efficiency of 40%. Such a performance change could be explained by the difference in the anaerobic sludge characteristics during the experiment. At an appropriate reflux ratio (e.g., 4), the sludge particles could fully contact the pollutants in the reactor, which favored the mass transfer and the degradation processes. Nevertheless, once the reflux ratio exceeded the optimal value, and increased up-flow velocity and a decreased residence time led to a disordered sludge distribution and a relatively poor pollutant degradation performance. These results suggested that one could tune the reactor performance by optimizing the reflux ratio.

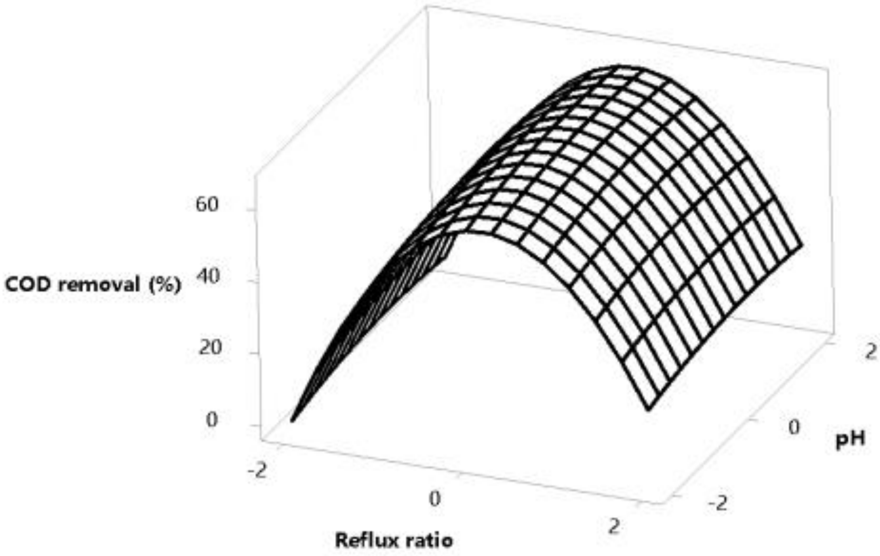

Figure 5-5. Surface Plot of COD removal (%) vs. pH, Reflux ratio.

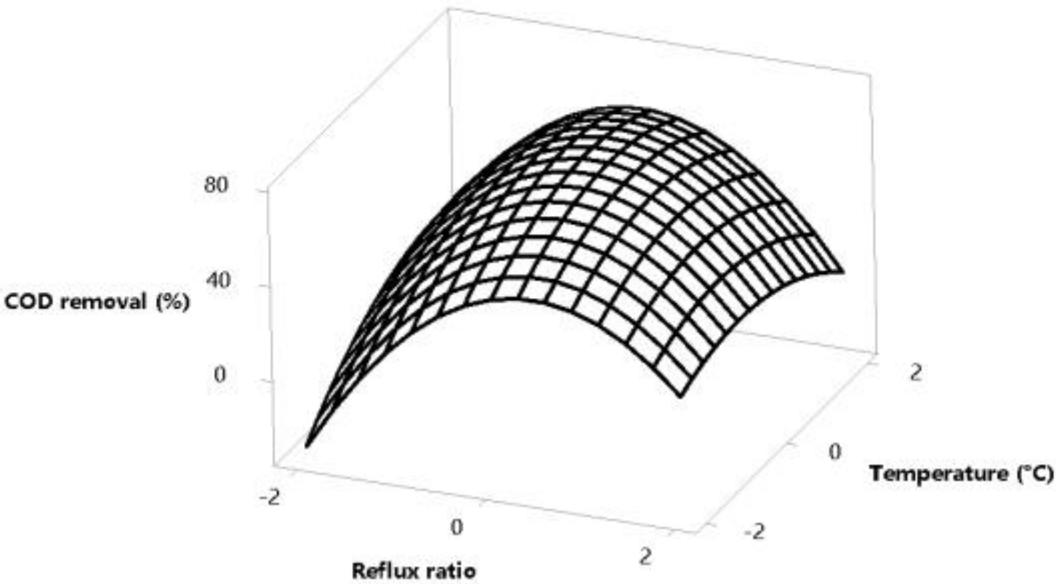

Figure 5-6. Surface Plot of COD removal (%) vs. Temperature (°C), Reflux ratio.

5.1.4 Effect of interaction time on COD removal

The interaction time plays a significant role for the systems based on the biological wastewater treatment mechanisms. The interaction time offered to the system ensures the successful completion of all the ongoing biochemical reactions, achieving partial or complete pollutants removal. The interaction time is termed hydraulic retention time (HRT). COD removal, the most preferred among the parameters used in determining the organics removal performance of a system, was found to be positively affected by the increase in HRT. The techniques with high HRT were observed to yield high COD removals compared to the systems being run at low HRT (Fig. 5-7). The facilitation of high HRT also offers sufficient interaction with the microbes, enzymes, and mucus present in the bedding, causing even more organic degradation. The observed results were found to agree with the previous study conducted by other researchers. Furthermore, it should also be mentioned that the constant increase of HRT may not always prove to be economical due to high floor space area requirements.

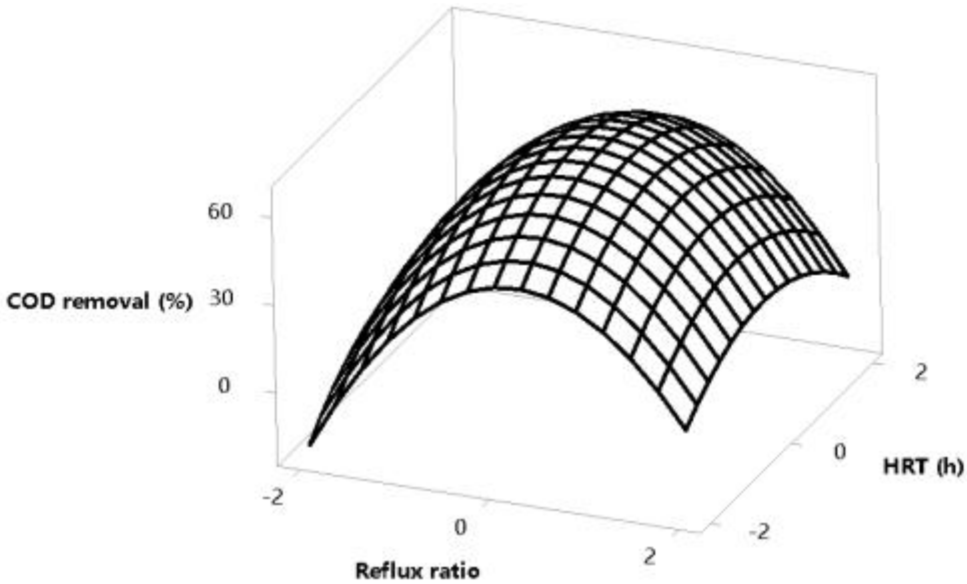

Figure 5-7. Surface Plot of COD removal (%) vs. HRT (h), Reflux ratio.

5.1.5 Effect of temperature on COD removal

An anaerobic reactor is developed and applied to treat industrial textile wastewater. The treatment performance is examined systematically, and the critical operational parameters are identified. The results demonstrated a stable and excellent COD removal of 64.99%. Interestingly, the bio-degradability was improved after the anaerobic reactor treatment. It was also observed that the application of higher hydraulic retention time positively impacts the treatment of industrial textile wastewater. COD removal increased when pH and temperature increased. The effects of the reactor reflux ratio on the COD removal under an optimized HRT of 8.5 h were observed.

5.2 STUDY ON THE OPERATIONAL EFFICIENCY OF VERTICAL FLOW LABYRINTH (VFL) DEVICE IN TREATING HUZHOU DYEING WASHING WASTEWATER

Due to the complex composition of dyeing washing wastewater, it contains dye wastewater. If it is not treated by professional technology, it will cause great damage to the ecological environment after discharge [33]. The use of biotechnology to treat sand washing wastewater has low cost, little secondary pollution to the environment, less sludge output, and does not require complex equipment [34]. It has good environmental and economic effects, and has become the most commonly used sand washing wastewater treatment technology. Under the biological anaerobic environment, the dye is decolorized through the reduction reaction catalyzed by the enzyme, and the azo is decomposed to generate the aromatic amine under the action of the bacterial group [35]. The use of anaerobic reactors can efficiently treat sand washing wastewater and reduce energy consumption. Generally, there are up-flow anaerobic sludge beds and anaerobic membrane bioreactors. In addition, general dyes have toxic and side effects on microorganisms in water, and aerobic treatment will cause swelling and floating of sludge. Generally, it can be used in combination with other treatment processes to improve biodegradability. With the addition of various new dyes and auxiliaries and the continuous improvement of printing and dyeing wastewater discharge standards, a single biodegradation technology to treat printing and dyeing wastewater can no longer meet the needs [36]. It is necessary to combine other pretreatment processes or advanced treatment technologies to meet current and future requirements Treatment needs of printing and dyeing wastewater. This study explores the effect of VFL-based biological treatment on sand washing wastewater in Huzhou, China.

5.2.1 Performance of biochemical oxygen demand removal

Five-day biochemical oxygen demand (BOD_5) is an important indicator that indirectly indicates the degree of water pollution by organic matter by the amount of dissolved oxygen consumed by microbial metabolism [37, 38]. It is mainly used to monitor the pollution of organic matter in water bodies. Generally, organic matter can be decomposed by microorganisms, but when microorganisms decompose organic compounds in water, they need to consume oxygen. If the dissolved oxygen in the water is not enough to supply the needs of microorganisms, the water body is in a state of pollution. In principle, the higher the ratio of BOD_5, the better the biodegradability of wastewater [39]. The water quality and pollutant components of printing and dyeing wastewater are mainly related to fiber types and processing techniques. Generally, the pH of printing and dyeing wastewater is 6.0-10.0, and the five-day biochemical oxygen demand (BOD_5) is 100-400 mg/L. In this experiment, during the period from August 1, 2021 to December 31, 2021, for the comprehensive improvement of the environment of the Wuxing children's clothing industry in Huzhou, China, the sand washing sewage in the supporting garden, the BOD_5 monitoring when treated by the VFL device is shown in Fig. 5-8. After VFL treatment, BOD_5 was effectively degraded. Specifically, influent (290.7 mg/L) > anaerobic stage (243.7 mg/L) > aerobic stage (90.6 mg/L) > anoxic stage (87.1 mg/L) > effluent (39.8 mg/L). After the sand washing wastewater passes through each section of the VFL unit, the average degradation rate of BOD_5 is as high as 86.3%. BOD_5 tends to be stable in each sampling section of VFL, and the change range is not large. It also indirectly reflects that the VFL device can degrade the organic matter in the sand washing wastewater very well. At the same time, it is speculated that the characteristic microorganisms of each stage are different. Haroun et al. used anaerobic fluidized bed to treat dye wastewater, and in their study, the BOD degradation rate reached 95% [40]. Pourbabaee et al. used newly isolated *Bacillus* sp. to treat dye wastewater. In their study, the untreated effluent samples had lower BOD values, while the treated samples showed an increase in initial BOD within 15 days, whereas in their study decreased after 20 days [41]. In order to better remove BOD, Han et al. combined the pilot-scale biological treatment (1000 m^3) with electron beam. The experimental results showed that the consumption of chemical reagents was reduced, the residence time was shortened, and the removal rate of COD and BOD was increased by 30%-40% [42]. In addition, Di and others designed a printing and dyeing wastewater treatment plan based on anaerobic-aerobic process, and used it to treat printing and dyeing wastewater [6]. The first-class discharge standard of Industrial Water Pollution Discharge Standard. O'Neill et al. used anaerobic-aerobic method to treat textile effluent, in their study, the maximum overall BOD removal was up to 99% [43]. In general, our device still has certain advantages.

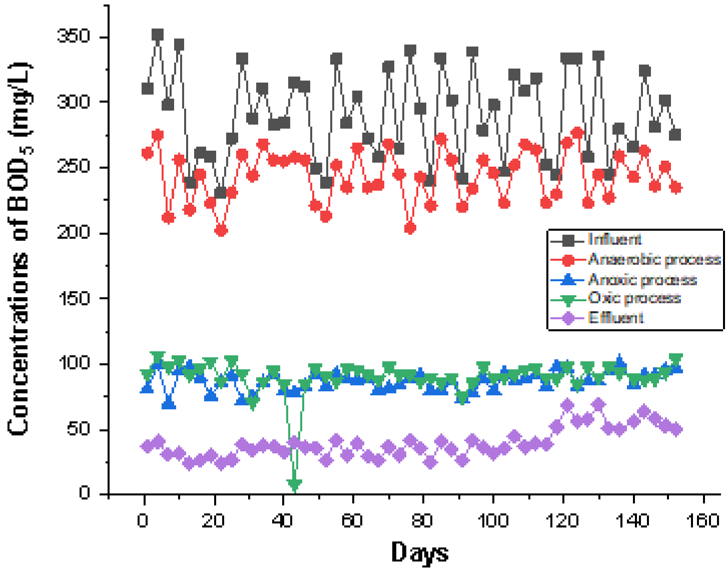

Figure 5-8. BOD_5 monitoring when VFL unit treats dyeing wastewater.

5.2.2 Performance of nitrogen removal

Dyestuff wastewater has now become one of the most serious factors threatening the safety of water bodies in China [44]. How to treat dye wastewater efficiently and quickly has become an urgent problem to be solved. Among them, ammonia nitrogen wastewater usually refers to wastewater containing NH_3 and NH_4^+ [45, 46]. The experimental operation time of this stage is from August to December 2021. The NH_4^+ monitoring of the sand washing wastewater treatment by the VFL unit is shown in Fig. 5-9. In general, during the sampling period, the NH_4^+ concentration at each stage was relatively stable, and it was speculated that the microbial community in the device was relatively stable. The average concentration of NH_4^+ in the influent (32.5 mg/L)> the average concentration of NH_4^+ in the anaerobic stage (16.1 mg/L)> the average concentration of NH_4^+ in the anoxic stage (7.3 mg/L)>the average concentration of NH_4^+ in the aerobic stage (4.4 mg/L)> the average concentration of NH_4^+ in the effluent (3.4 mg/L). The degradation rate of NH_4^+-N in the aerobic stage reaches 86.5%, and the degradation rate in the effluent reaches 90%, and the removal effect is very obvious. The removal of ammonia nitrogen is mainly converted into nitrate nitrogen by the action of nitrifying bacteria in the aerobic stage.

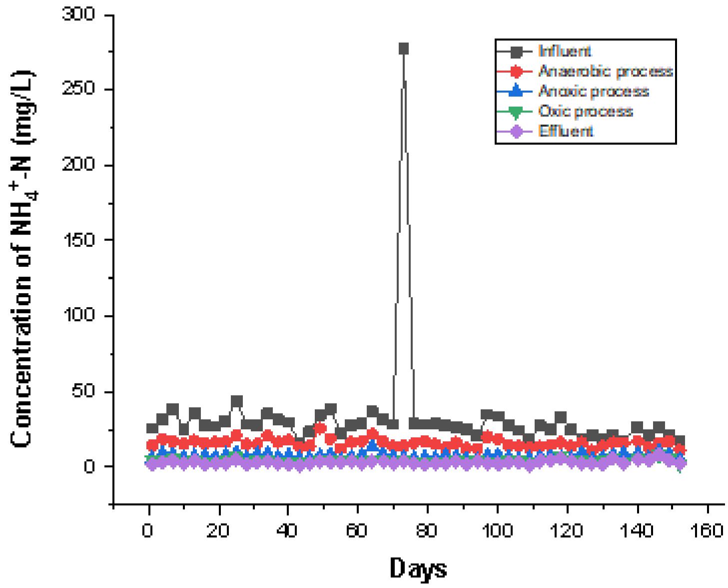

Figure 5-9. Monitoring of ammonia nitrogen in dyeing wastewater treatment by VFL.

The traditional nitrification and denitrification process is to first convert NH_4^+-N into NO_3^--N through nitrification, and then reduce NO_3^--N to N_2 through denitrification to achieve the purpose of total nitrogen removal [47]. In this experiment (Fig. 5-10), the change of NO_3^--N concentration in the VFL device is opposite to the change of NH_4^+-N, that is, the average concentration of NO_3^--N in the effluent (1.69mg/L)> the NO_3 in the aerobic stage --N average concentration (1.63mg/L)> NO_3^--N average concentration in anoxic stage (0.40mg/L)> NO_3^--N average concentration in anaerobic stage (0.18mg/L)> influent NO_3^--N average concentration (0.08mg/L). As compared, Laizer et al. proposed that domestic wastewater as a carbon source to enhance treatment of dyeing wastewater [48]. However, in their study, NH_4^+-N and NO_3^--N increased at 49 and 87%, respectively.

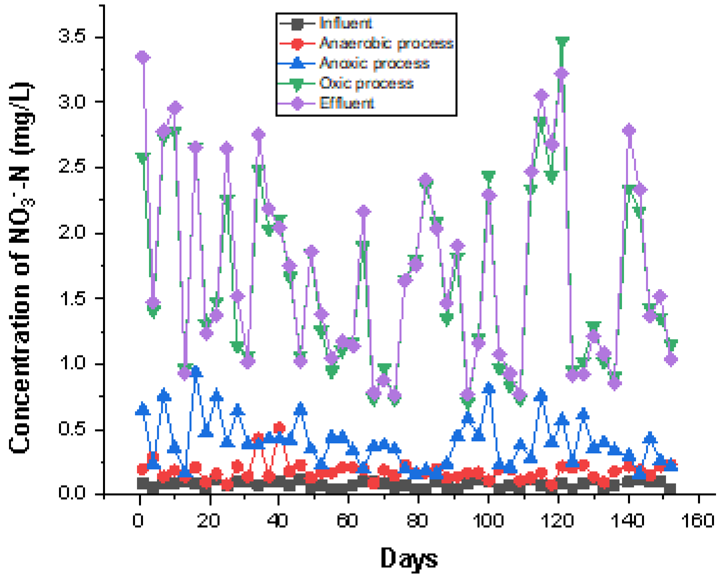

Figure 5-10. Monitoring of nitrate nitrogen when VFL unit treats dyeing wastewater.

In this experiment (Fig. 5-11), the variation law of total nitrogen is the same, that is, the average concentration of total nitrogen in the influent (31.6 mg/L) > the average concentration of total nitrogen in the anaerobic stage (17.6 mg/L) > the total nitrogen in the anoxic stage The average concentration of nitrogen (10.5 mg/L)> the average concentration of total nitrogen in the aerobic stage (7.0 mg/L)> the average concentration of total nitrogen in the effluent (6.3 mg/L). Good nitrification is the premise of total nitrogen removal, and nitrification is mainly completed in the aerobic section [49]. The average removal rate of total nitrogen in the anaerobic stage was 44.3%, the average removal rate of total nitrogen in the anoxic stage was 66.8%, the average removal rate of total nitrogen in the aerobic stage was 77.8%, and the average removal rate of total nitrogen in the effluent was 80%. Overall, the total nitrogen removal rate was better during the long-term stable operation. In addition, the total nitrogen concentration in the influent stage was between 20-48 mg/L, and the fluctuation range was enlarged. In contrast, the total nitrogen concentration in the effluent stage was between 3.1-10.2 mg/L, with a small fluctuation range and relatively stable. In this experiment, it is speculated that NO_3^--N affects the removal of total nitrogen. The content of macromolecular nitrogen-containing organic substances such as azide and azo in the wastewater of the printing and dyeing industry is relatively high. It is difficult for traditional anaerobic/anoxic/aerobic processes to break the chain into small molecular organic substances and further mineralize them, so the removal capacity is very limited. The removal of organic nitrogen has also become a difficulty in removing total nitrogen from wastewater in the printing and dyeing wastewater industry.

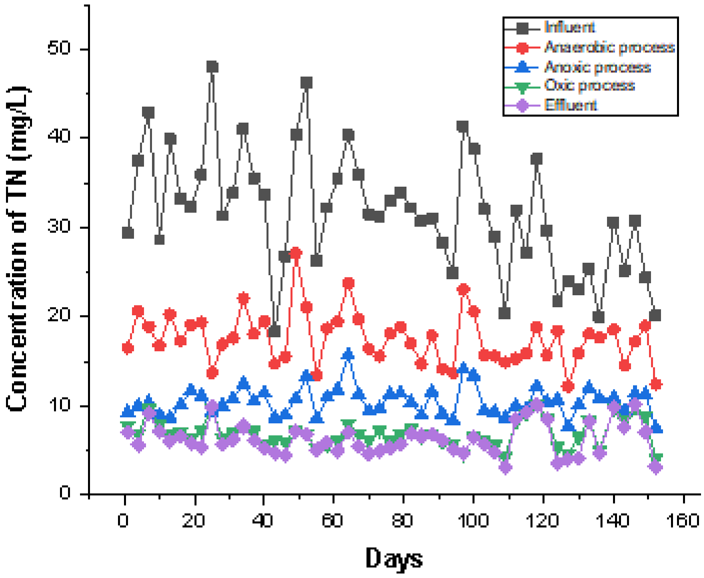

Figure 5-11. Monitoring of total nitrogen when VFL unit treats dyeing wastewater.

5.2.3 Performance pf phosphorus removal

In addition to nitrogen, the excessive discharge of phosphorus is also one of the main reasons for eutrophication of water bodies [50, 51]. For this reason, the concentration of phosphorus is strictly limited in the wastewater discharge standards of various industries [52]. The commonly used biological phosphorus removal technology is to alternately operate in anaerobic-aerobic or anaerobic-anoxic units, and utilize the characteristics of anaerobic phosphorus release and aerobic phosphorus absorption of phosphorus-accumulating microorganisms to overabsorb in anaerobic or anoxic units. Phosphorus in sewage is discharged in the form of excess sludge to achieve phosphorus removal from sewage [53]. The advantage of the biological phosphorus removal process is that there is no need to add external chemicals, and it will not cause secondary pollution and changes in water quality indicators. As shown in Fig. 5-12, the total phosphorus was monitored when the VFL unit treated the sand washing wastewater. The results showed that the average concentration of total phosphorus in the influent section was 2.4 mg/L, the average concentration of total phosphorus in the anaerobic section was 3.2 mg/L, the average concentration of total phosphorus in the anoxic section was 1.6 mg/L, and the average concentration of total phosphorus in the aerobic section was 1.6 mg/L. The average concentration of phosphorus was 0.7 mg/L, and the average concentration of total phosphorus in the effluent section was 0.8 mg/L. The total phosphorus degradation rate in the effluent reached 66.7%. Rondon et al. used a membrane bioreactor to treat dye wastewater. The membrane bioreactor was composed of two anoxic reactors and one gas-explosion membrane reactor. The results showed that when the hydraulic retention time was 74.4 hours, the phosphorus removal rate reached 73% [54]. Liu et al. developed a gas-explosion biofilter filled with oyster shells to treat urban domestic sewage, and the average removal rate of total phosphorus by oyster shell biofilters reached 70% [55]. In addition, according to "Anaerobic Hypoxic and Aerobic Activated Sludge Process Engineering Technical Specifications" (hJ579-2010), the BOD_5/TP value of the influent water needs to be ≥17 for biochemical phosphorus removal. As shown in Figure 5, the BOD_5/total phosphorus (TP) of this experiment was always greater than 17. Specifically, the average value of BOD_5/TP in the influent section is 124.3, the average value of BOD_5/TP in the anaerobic section is 77.3, the average value of BOD_5/TP in the anoxic section is 56.4, and the average value of BOD_5/TP in the aerobic section is 56.4. The average value of 132.5, and the average value of BOD_5/TP in the outlet section reached 56.1. In addition, the BOD_5/TP ratio of each section of the VFL device is relatively stable.

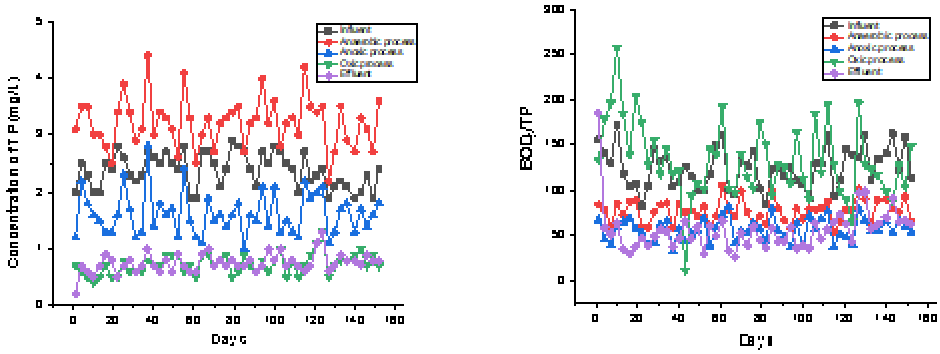

Figure 5-12. Monitoring of total phosphorus during sand washing wastewater treatment by VFL.

5.2.4 Changes in wastewater pH

The pH of the solvent can alter the pollutants state and affect the removal performance [56]. In addition, pH can also influence the metabolic activity of substrates [57]. As known, large amounts of salts such as sodium sulfate, sodium chloride, and sodium nitrate are utilized in the dye manufacturing industries and in the dye-consuming industries, besides, sodium hydroxide is widely used to enhance the pH to the alkaline range [58]. Moreover, in the design and operation of most biochemical treatments, pH is critical and affects the enzymatic systems responsible for microbial activity [59]. Therefore, in wastewater treatment, the effect of pH on enzymatic activity is further translated into the effect on the corresponding microorganisms involved. Both above and below the optimum pH will result in a decrease in enzyme activity, which in turn affects the reaction rate. In general, optimal growth of bacteria requires near-neutral pH. The dominant bacteria in the dye-containing wastewater are suitable for growth in a neutral environment with a pH equal to 7, which is conducive to the growth, reproduction and metabolism of microorganisms. Therefore, this study also monitored the pH of the sand washing wastewater treated by the VFL unit. As shown in Fig. 5-13, the pH changes in the VFL device are not large and relatively stable. Specifically, the average pH of the influent section is 6.6, the average pH of the anaerobic section is 6.3, the average pH of the anoxic section is 6.8, the average pH of the aerobic section is 6.6, and the average pH of the effluent section is 6.8. It indirectly shows that the neutral environment in the device can maintain the metabolism and growth of microorganisms, stabilize the activity of enzymes, and thus will not affect the degradation of wastewater. Selvakumar et al. utilized the white-rot fungus *Ganoderma lucidum* to biodegrade printing and dyeing wastewater, and in their study, pH 6.6 was the optimal condition [60].

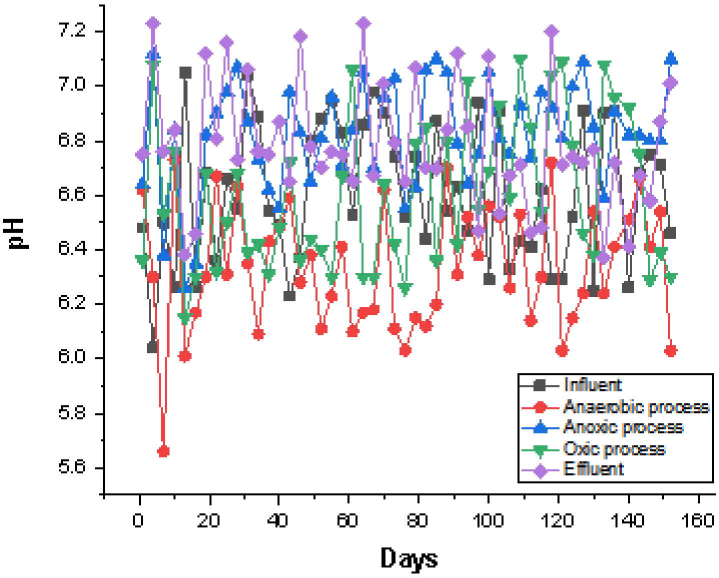

Figure 5-13. Monitoring of pH during dyeing wastewater treatment by VFL.

5.2.5 Summary

1) After VFL treatment, BOD_5 was effectively degraded. Influent (290.7 mg/L)> anaerobic section (243.7 mg/L)> aerobic section (90.6 mg/L)> anoxic section (87.1 mg/L)> effluent (39.8 mg/L). After the sand washing wastewater passes through each section of the VFL unit, the average degradation rate of BOD_5 is as high as 86.3%. BOD_5 tends to be stable in each sampling section of VFL, and the change range is not large.

2) During the sampling period, the NH_4^+ concentration at each stage was relatively stable. The average concentration of NH_4^+ in the influent (32.5 mg/L)>the average concentration of NH_4^+ in the anaerobic stage (16.1 mg/L)>the average concentration of NH_4^+ in the anoxic stage (7.3 mg/L)>the average concentration of NH_4^+ in the aerobic stage (4.4 mg/L)> the average concentration of NH_4^+ in the effluent (3.4 mg/L).

3) The change of NO_3^--N concentration in the VFL device is opposite to the change of NH_4^+-N, that is, the average concentration of NO_3^--N in the effluent (1.69mg/L) > the average concentration of NO_3^--N in the aerobic section (1.63 mg/L)> the average concentration of NO_3^--N in the anoxic stage (0.40mg/L)> the average concentration of NO_3^--N in the anaerobic stage (0.18mg/L)> the average concentration of NO_3^--N in the influent (0.08mg/L).

4) The average removal rate of total nitrogen in the anaerobic stage is 44.3%, the average removal rate of total nitrogen in the anoxic stage is 66.8%, the average removal rate of total nitrogen in the aerobic stage is 77.8%, and the average removal rate of total nitrogen in the effluent is 80%. Overall, the total nitrogen removal rate was better during the long-term stable operation.

5) The average concentration of total phosphorus in the influent section is 2.4 mg/L, the average concentration of total phosphorus in the anaerobic section is 3.2 mg/L, the average concentration of total phosphorus in the anoxic section is 1.6 mg/L, and the average concentration of total phosphorus in the aerobic section is 1.6 mg/L. The average concentration was 0.7 mg/L, and the average concentration of total phosphorus in the effluent section was 0.8 mg/L. The total phosphorus degradation rate in the effluent reached 66.7%.

6) The change of pH in the VFL device is not large and relatively stable. The average pH of the influent section was 6.6, the average pH of the anaerobic section was 6.3, the average pH of the anoxic section was 6.8, the average pH of the aerobic section was 6.6, and the average pH of the effluent section was 6.8.

5.3 STUDY ON THE TREATMENT OF HUZHOU DYEING WASTEWATER BY TREADITIONAL ANAEROBIC ANOXIC AEROBIC PROCESS

In the anoxic/aerobic (A/O) process, the anoxic tank is at the front end, making full use of the organic matter in the influent as a carbon source to denitrify and denitrify the NO_3^--N in the reflux mixture [61], and the aerobic tank is at the back end, the nitrification reaction and the removal of residual organic matter are carried out [62]. The advantage of this process is that the organic matter in the influent can be fully utilized as the carbon source for denitrification, the alkalinity generated by denitrification can be supplemented to the aerobic digestion tank in the latter stage, and the organic load of the aerobic tank can be reduced, which is conducive to the enrichment of chemoautotrophic nitrifying bacteria. Anaerobic/Anaerobic/Aerobic (AAO) process is to add an anaerobic stage before the A/O system. Comparison experiment between AAO process and AO process by Zhang et al. showed that AAO process is superior to AO process in terms of NH_4^+-N removal and denitrification, especially in terms of denitrification rate, AAO process is twice that of

AO process [63]. AAO process is a simple sewage treatment process with simultaneous biological nitrogen and phosphorus removal functions. Due to its simple structure, short hydraulic retention time, less sludge expansion and mature design and operation experience, it has become the most widely used in the world. One of a wide range of wastewater treatment processes. In this chapter, the AAO process is selected as a control to explore the effect of AAO on sand washing wastewater.

5.3.1 Performance of BOD_5 removal

Figure 5-14 shows the monitoring of BOD_5 concentration in sand washing wastewater treated by AAO process for a long time. The sampling period is between August 1, 2021 and December 31, 2021. In this experiment, the fluctuation range of BOD_5 was small in the effluent stage, the aerobic stage, and the anoxic stage; however, the fluctuation range of BOD_5 was larger in the influent and anaerobic stage. The average concentration of BOD_5 in the influent section is 290.7 mg/L, the average concentration of BOD_5 in the anaerobic section is 262.4 mg/L, the average concentration of BOD_5 in the anoxic section is 96.1 mg/L, and the average concentration of BOD_5 in the aerobic section is 100.1 mg/L. L, the average concentration of BOD_5 in the effluent section was 43.9 mg/L. The BOD_5 degradation rate in the effluent section reached 84.9%. Hao et al. combined a sequencing batch reactor with iron filings filtration to treat printing and dyeing wastewater. When the concentration of BOD_5 influent was in the range of 200-400 mg/L, the new combined process could effectively remove 90% of BOD_5 [64].

It was found that there was a relationship between the COD and BOD [65]. The ratio of BOD_5 to COD, that is, B/C, is usually used to reflect the biodegradability of wastewater, which is currently the most classic and commonly used water quality index evaluation method [66]. Among them, BOD_5 represents the amount of degradable organic matter, and COD represents the total amount of organic matter [67]. The relationship between the biodegradability of wastewater and B/C is that when B/C is greater than 0.45, it is suitable for biological treatment; when B/C is between 0.2 and 0.45, it needs to be determined by experiment; if B/C is less than 0.2, biochemical treatment is used. is more difficult [68]. But in general, only when B/C is greater than 0.3, it can be effectively treated by biological method. Therefore, in this experiment, BOD_5/COD is also listed as a detection and analysis index, and the results of the biodegradability of sand washing wastewater by the AAO process as a control process are investigated. As shown in Figure 5-14, the ratio of BOD_5 to COD in the inlet section is always greater than 0.3 when the AAO process treats sand washing wastewater for a long time, which indicates that the sand washing wastewater can be effectively treated by biological methods. In an experiment to improve the biological treatment capacity, the ozone oxidation process was combined as a pretreatment with anoxic-aerobic activated sludge, and the experimental results showed that the B/C of the wastewater was increased to 0.38 [69]. As compared, in a study by Selvakumar et al. [60], treatment of textile dye wastewater was carried in a batch reactor using *Ganoderma lucidum*. From the results, under the pH 6.6, agitation speed 200 rpm, and temperature 26.5 ℃, the maximum COD reduction was 90.3%.

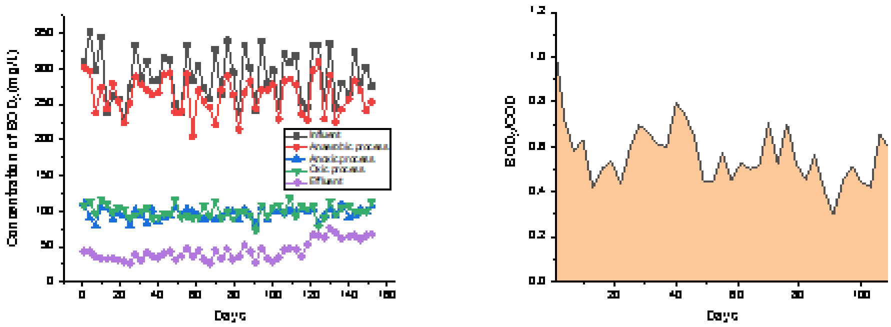

Figure 5-14. Monitoring of BOD_5 in sand washing wastewater treated by AAO process for a long time.

5.3.2 Performance of nitrogen removal

The AAO process is evolved from the traditional AO process. On the basis of the traditional biological denitrification principle, the operation and application methods are adjusted to cope with the sewage treatment conditions of different water quality and quantity. The AAO method is mainly used in the treatment of domestic sewage with large flow and low concentration. In general, the advantages of the traditional biological denitrification process are that various types of microorganisms with different functions grow and proliferate in different reactors, the growth environment conditions are suitable, and the reaction speed is fast and thorough. But at the same time, there are also the following problems: 1. The biological concentration of nitrifying bacteria with a long generation cycle is not high in the activated sludge tank. 2. It is necessary to add alkali to neutralize the acid produced by the nitrification reaction, which increases the treatment cost and may cause secondary pollution. 3. It is necessary to carry out sludge return and nitrification liquid return at the same time to maintain the concentration of microorganisms, which increases the operating cost and power consumption. As shown in Fig. 5-15, the NH_4^+-N during the long-term treatment of sand washing wastewater by the AAO process was monitored. The average concentration of NH_4^+-N in the inlet section is 32.5 mg/L, the average concentration of NH_4^+-N in the anaerobic section is 16.8 mg/L, the average concentration of NH_4^+-N in the anoxic section is 8.5 mg/L, and the average concentration of NH_4^+-N in the aerobic section is 8.5 mg/L. The average concentration of NH_4^+-N in the outlet section is 3.9 mg/L. Compared with the concentration of NH_4^+-N in the influent, the NH_4^+-N in the effluent was effectively degraded, and the degradation rate reached 88%, and the degradation effect was better.

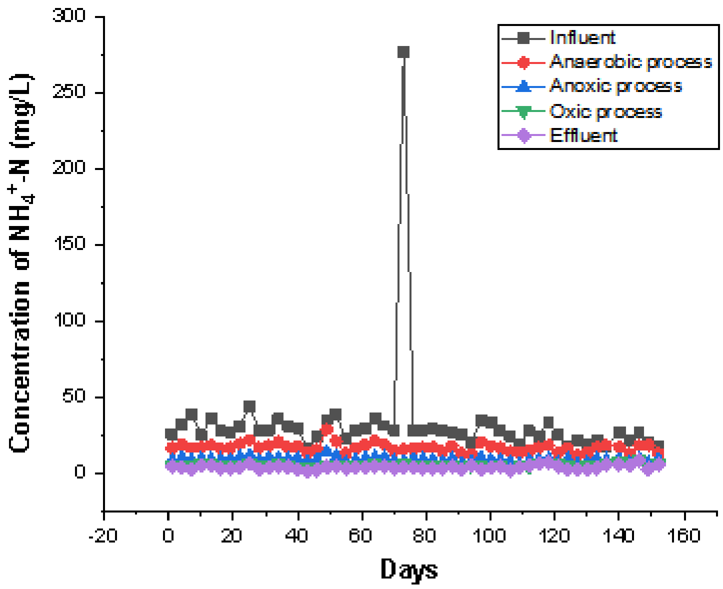

Figure 5-15. Monitoring of ammonia nitrogen in long-term sand washing wastewater treatment by AAO process.

As far as we know, nitrification refers to the process of converting NH_4^+-N in sewage into NO_3^--N under the action of nitrifying bacteria under aerobic conditions. The reaction proceeds in two steps, firstly, NH_4^+-N is converted into NO_2^--N by ammonia oxidizing bacteria (AOB), and then NO_2^--N is converted by nitrite oxidizing bacteria (NOB) Converted to NO_3^--N. AOB and NOB are collectively referred to as nitrifying bacteria, which are aerobic autotrophic bacteria. Nitrification is the first step in denitrification, and its performance has a direct impact on the denitrification efficiency. In this experiment (Fig. 5-16), the average concentration of NO_3^--N in the influent section was 0.08 mg/L, the average concentration of NO_3^--N in the anaerobic section was 0.15 mg/L, and the average concentration of NO_3^--N in the anoxic section was 0.34 mg/L. The average concentration of NO_3^--N in the aerobic section is 1.29 mg/L, and the average concentration of NO_3^--N in the effluent section is 1.32 mg/L. NO_3^--N effluent concentration is higher than influent concentration. NO_3^--N changes very smoothly in the influent section, anaerobic section and anoxic section. On the contrary, NO_3^--N fluctuates greatly in the aerobic section and the effluent section. Combined with the above discussion, it is speculated that the reason for the rise of NO_3^--N is that nitrosobacteria and nitrifying bacteria convert NH_4^+-N to NO_3^--N through aerobic nitrification. This is consistent with the VFL device changes in the previous chapter.

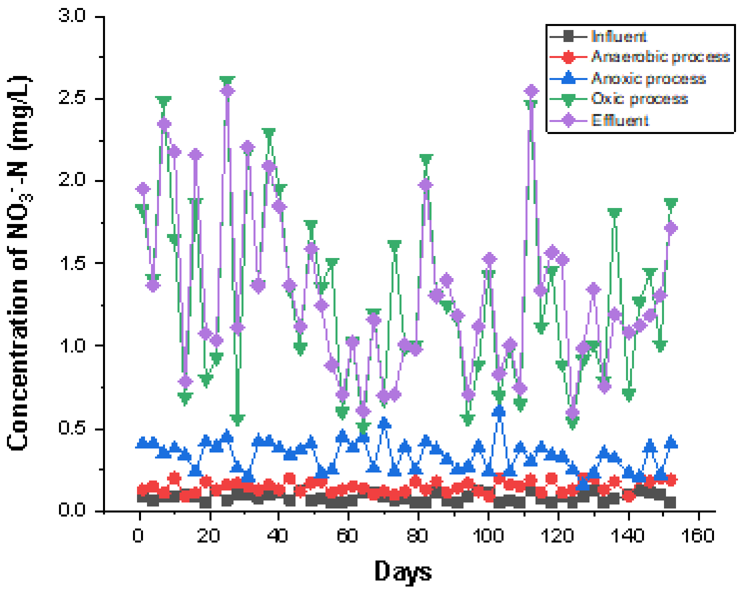

Figure 5-16. Monitoring of nitrate nitrogen in long-term sand washing wastewater treatment by AAO process.

In addition, the total nitrogen was monitored synchronously in this experiment, and the results are shown in Fig. 5-17. The change trend of total nitrogen is the same as that of NH_4^+-N. The average concentration of total nitrogen in the influent section was 31.6 mg/L, the average concentration of total nitrogen in the anaerobic section was 22.3 mg/L, the average concentration of total nitrogen in the anoxic section was 9.9 mg/L, and the average concentration of total nitrogen in the aerobic section was is 7.5 mg/L, and the average concentration of total nitrogen in the effluent section is 6.5

mg/L. The total nitrogen in the effluent section was well degraded, and the degradation rate reached 79.4%. It is slightly lower than the total nitrogen degradation rate of VFL treatment of sand washing wastewater in the previous chapter. One factor that affects the degradation of total nitrogen is the rise of NO_3^--N. It is worth noting that refractory organic nitrogen is also an important factor affecting the compliance of total nitrogen, and refractory organic nitrogen is the focus and difficulty of engineering treatment.

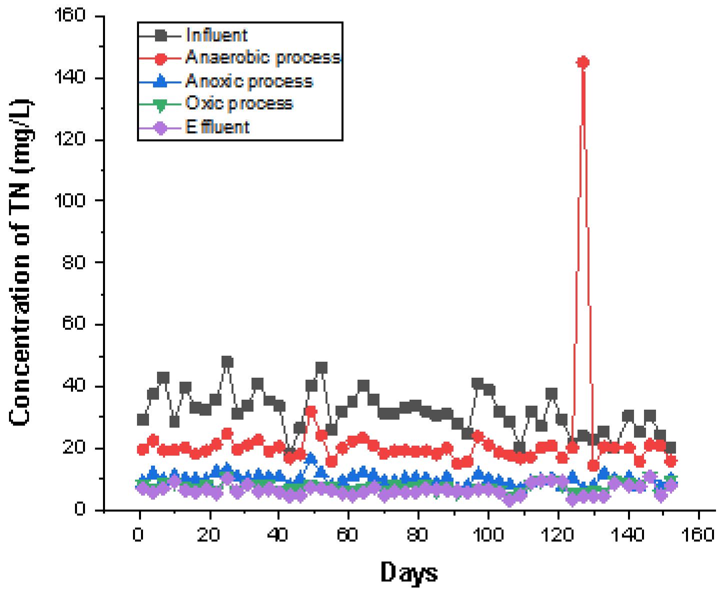

Figure 5-17. Monitoring of total nitrogen in sand washing wastewater treated by AAO process for a long time.

5.3.3 Performance of P removal

As shown in Fig. 5-18, the average concentration of total phosphorus in the influent section is 2.4 mg/L, the average concentration of total phosphorus in the anaerobic section is 3.0 mg/L, the average concentration of total phosphorus in the anoxic section is 1.6 mg/L, and the average concentration of total phosphorus in the aerobic section is 1.6 mg/L. The average concentration of total phosphorus in the effluent section was 0.8 mg/L, and the average concentration of total phosphorus in the effluent section was 0.8 mg/L. The degradation rate of phosphorus in the effluent section reached 66.7%. The degradation rate of total phosphorus in sand washing wastewater by the VFL device in the previous chapter is the same as that in the previous chapter. However, it is worth noting that the total phosphorus content of the anaerobic section exceeds the total phosphorus content of the influent section. In the AAO process, the sewage passes through the anaerobic tank, the anoxic tank and the aerobic tank in turn, and the return sludge will bring a part of the nitrate back to the anaerobic area. The existence of nitrate makes the denitrifying bacteria preferentially compete for the carbon source of the influent water, thus seriously affecting the phosphorus release efficiency of phosphorus accumulating bacteria, which in turn affects the phosphorus removal efficiency of the system. Studies have shown that denitrifying phosphorus accumulating bacteria can simultaneously denitrify and absorb phosphorus using nitrite as an electron acceptor, thereby reducing the accumulation of nitrite in the system and improving phosphorus removal efficiency [70]. Therefore, in order to improve the nitrogen and phosphorus removal efficiency and ensure that the effluent nitrogen and phosphorus indicators meet the standards, it is often necessary to add chemicals, which increases the treatment cost of the sewage treatment plant [71].

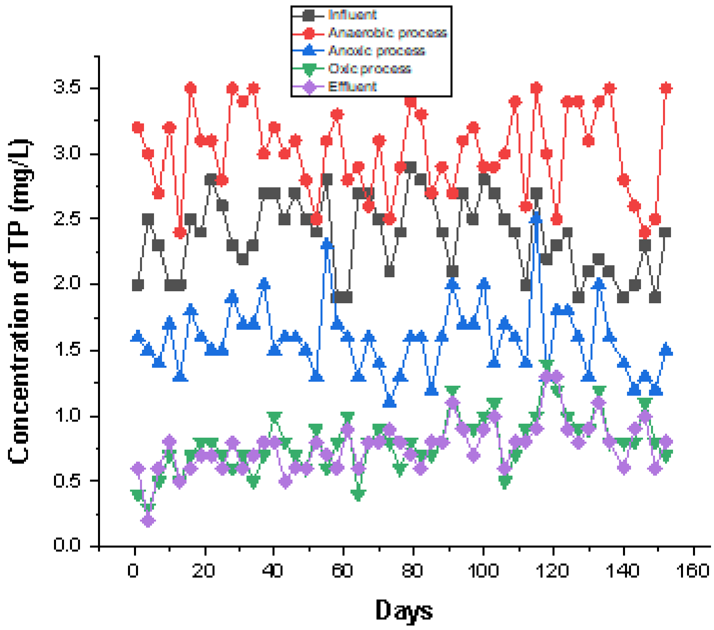

Figure 5-18. Monitoring of total phosphorus in sand washing wastewater treated by AAO process for a long time.

5.3.4 Changes in wastewater pH

pH value is an important factor affecting the biological treatment of wastewater [72]. The change of pH value will cause changes in the surface charge of microorganisms, thereby affecting the absorption and degradation of NH_4^+-N by microorganisms. In addition, too high pH value will inhibit the activity of nitrifying bacteria and affect the nitrification process, thereby reducing the removal rate of NH_4^+-N in the composite bioreactor. pH can also alter the nutrient and availability in the microbial growth environment, enhancing the toxicity of harmful substances, thereby hindering the effective degradation of pollutants by microorganisms. Therefore, the monitoring of pH value is very necessary. In this experiment, the monitoring of pH for a long time is shown in Fig. 5-19. The average pH of the influent section was 6.6, the average pH of the anaerobic section was 6.2, the average pH of the anoxic section was 6.7, the average pH of the aerobic section was 6.5, and the average pH of the effluent section was 6.6. The pH value of the influent water of the first-stage aerobic microbial contact reactor is about 7, which is suitable for the growth of decarbonizing heterotrophic bacteria, nitrifying bacteria and denitrifying bacteria. The activity of nitrifying bacteria was the strongest when the pH was 7.0-7.8 and 7.7-8.4. However, in the process of digestion, nitrifying bacteria will consume the alkalinity in the wastewater and move the pH value to the acid direction. When the pH value of the effluent is seriously reduced, it will not only affect the nitrification reaction, but also will not meet the requirements of sewage discharge standards.

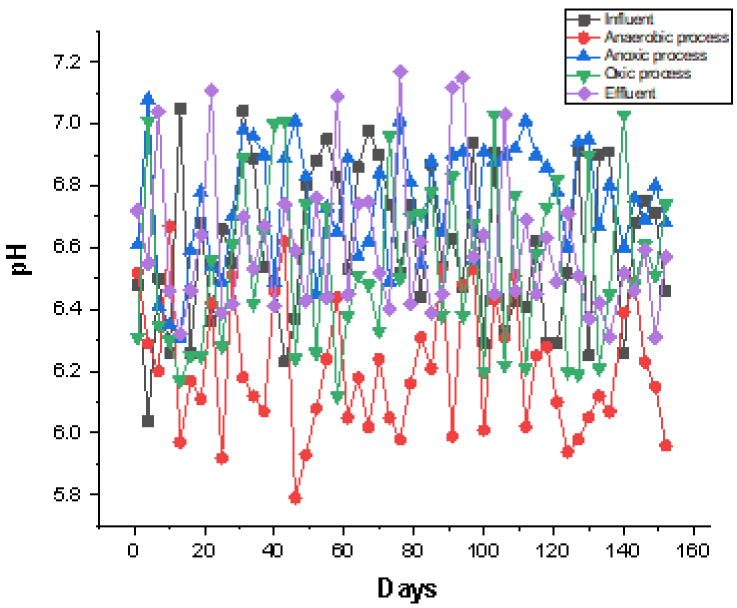

Figure 5-19. pH monitoring of sand washing wastewater treated by AAO process for a long time.

5.3.5 Summary

1) The fluctuation range of BOD_5 is small in the effluent stage, the aerobic stage, and the anoxic stage; however, the fluctuation range of BOD_5 is larger in the influent and anaerobic stage. The average concentration of BOD_5 in the influent section is 290.7 mg/L, the average concentration of BOD_5 in the anaerobic section is 262.4 mg/L, the average concentration of BOD_5 in the anoxic section is 96.1 mg/L, and the average concentration of BOD_5 in the aerobic section is 100.1 mg/L. L, the average concentration of BOD_5 in the effluent section was 43.9 mg/L. The BOD_5 degradation rate in the effluent section reached 84.9%. When the AAO process treats sand washing wastewater for a long time, the ratio of BOD_5 to COD in the inlet section is always greater than 0.3, which indicates that the sand washing wastewater can be effectively treated by biological methods.

2) The average concentration of NH_4^+-N in the inlet section is 32.5 mg/L, the average concentration of NH_4^+-N in the anaerobic section is 16.8 mg/L, the average concentration of NH_4^+-N in the anoxic section is 8.5 mg/L, and the average concentration of NH_4^+-N in the aerobic section is 8.5 mg/L. The average concentration of NH_4^+-N is 5.1 mg/L, and the average concentration of NH_4^+-N in the effluent section is 3.9 mg/L. Compared with the concentration of NH_4^+-N in the influent, the NH_4^+-N in the effluent was effectively degraded, and the degradation rate reached 88%, and the degradation effect was better.

3) The average concentration of NO_3^--N in the inlet section is 0.08 mg/L, the average concentration of NO_3^--N in the anaerobic section is 0.15 mg/L, and the average concentration of NO_3^--N in the anoxic section is 0.34 mg/L. The average concentration of NO_3^--N in the aerobic section was 1.29 mg/L, and the average concentration of NO_3^--N in the effluent section was 1.32 mg/L. NO_3^--N effluent concentration is higher than influent concentration. NO_3^--N changes very smoothly in the influent section, anaerobic section and anoxic section. On the contrary, NO_3^--N fluctuates greatly in the aerobic section and the effluent section.

4) The average concentration of total phosphorus in the influent section is 2.4 mg/L, the average concentration of total phosphorus in the anaerobic section is 3.0 mg/L, the average concentration of total phosphorus in the anoxic section is 1.6 mg/L, and the average concentration of total phosphorus in the aerobic section is 1.6 mg/L. The average concentration is 0.8 mg/L, and the average concentration of total phosphorus in the effluent section is 0.8 mg/L. The degradation rate of

phosphorus in the effluent section reached 66.7%. The degradation rate of total phosphorus in sand washing wastewater by the VFL device in the previous chapter is the same as that in the previous chapter.

5) After long-term monitoring of pH, it was found that the average pH of the influent section was 6.6, the average pH of the anaerobic section was 6.2, the average pH of the anoxic section was 6.7, and the average pH of the aerobic section was 6.5. The average pH of the effluent section was 6.6.

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Chapter 6

STUDY ON MICROBIAL COMMUNITY OF HUZHOU DYEING WASTEWATER TREATED BY VERTICAL FLOW LABYRINTH (VFL) DEVICE AND PROCESS

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CHAPTER 6: STUDY ON MICROBIAL COMMUNITY OF HUZHOU DYEING WASTEWATER TREATED BY VERTICAL FLOW LABYRINTH (VFL) DEVICE AND PROCESS

Biological treatment technology based on activated sludge microbial community has been widely used in water treatment. However, the active microbial community of activated sludge is highly complex [1-3], and different types of microorganisms interact with each other through material, energy, and information exchange—effects, such as competition, symbiosis, parasitism, etc. Similarly, the removal of pollutants does not only rely on a single microorganism but requires the synergy between multiple microorganisms [4]. This complex ecological relationship can be expressed as a microbial molecular environmental network, with species as nodes and species interaction as links [5, 6]. For example, the composition and state of the denitrifying microbial community directly affect the pathway and efficiency of nitrogen biotransformation in wastewater [7, 8]. A comprehensive understanding of microbial community structure and function can provide a microbiological basis for the diagnosis and targeted regulation of specific water treatment processes [9, 10]. However, previous studies on microorganisms in wastewater treatment processes were based on high-throughput sequencing analysis of 16S rRNA genes [11]. However, due to the bias brought by the amplification process and the difficulty in obtaining results directly from the perspective of functional genes, accurate reactions cannot be accepted. The actual community structure and functional characteristics in complex systems limit researchers' understanding of microbial structure and function in specific water treatment processes. In 1998, Handelsman first proposed the concept of "metagenome" [12]. Metagenome was initially developed as a tool for the functional and sequential analysis of microbial genomes contained in environmental samples and is now set to apply modern molecular biology techniques to obtain target microbial communities from the environment [13, 14]. In this study, the metagenome was used to compare and analyze the microbial community changes and gene functions of conventional AAO process and VFL device in the treatment of sand-washing wastewater by constructing a VFL device.

6.1 Rarefaction curve

The rarefaction curve is a method that adjusts for differences in library sizes across samples to aid comparisons of alpha diversity [15-17], and can indicate whether the sampling size of the sample is reasonable, and the rarefaction curve is constructed based on the sequence read length and the number of microbial species obtained by random sampling, as shown in Fig. 6-1. When the number of randomly sampled sequences is more significant than 10,000, the growth trend of species abundance slows down [18]. It begins to become flat, indicating that with the increase of the random sample number, more new species will not be generated, and the sampling is qualified. While the rarefaction curve does not reach saturation, which is consistent with results in the literature, an increase in the number of samples sequenced does not affect the rise in the number of microbial communities and does not tend to fully saturate [19, 20]. Compared with the AAO process, the confidence of the bacterial community structure in the VFL device is higher, and it can more realistically reflect the bacterial community of the sample.

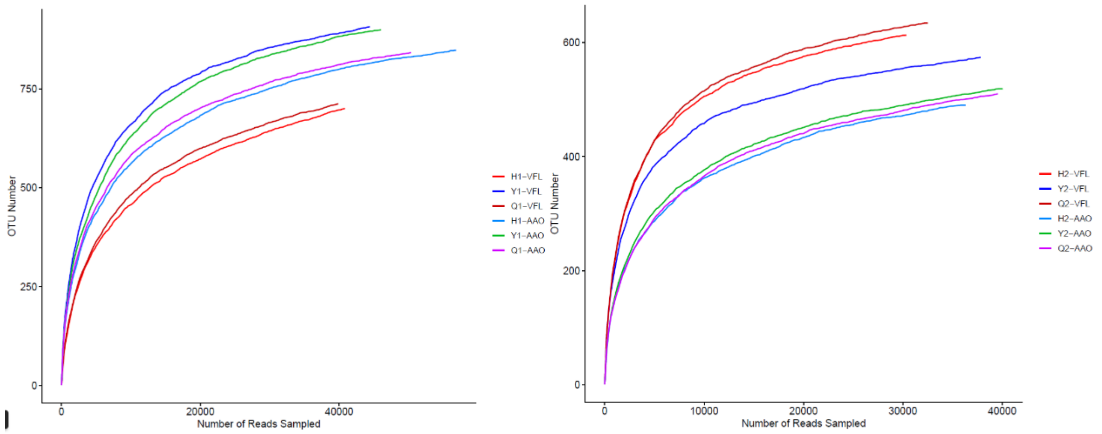

Figure 6-1. Dilution curves of two batches of samples from 2021.8.12 and 2021.8.20.

6.2 Alpha diversity analysis of overall microbial community structure

The Alpha diversity index is the diversity of microbial populations within an independent sample, which can be evaluated according to the distribution law at the level of annotated species [21-23]. The Alpha diversity index of the samples is shown in Table 6-1. The Chao index values of the samples collected on 2021.8.12 are: H1-AAO (955.462), H1-VFL (847.777), Q1-AAO (926.150), Q1-VFL (867.419), Y1-AAO (972.598) and Y1-VFL (1005.039). The Chao index of the anaerobic section of the VFL unit is greater than that of the AAO process [24]. The corresponding Chao index values of the samples collected on 2021.8.20 are H2-AAO (595.208), H2-VFL (692.596), Q2-AAO (633.667), Q2-VFL (712.981), Y2-AAO (625.047) and Y2 - VFL (663.553). The Chao index of each stage of the VFL device is greater than that of the AAO process. The Shannon index values of the samples collected on 2021.8.12: H1-AAO (4.445), H1-VFL (2.731), Q1-AAO (4.525), Q1-VFL (2.950), Y1-AAO (4.579) and Y1-VFL (4.821). The adoption of this batch is consistent with the Chao index. The Shannon index of the aerobic and anoxic sections of the VFL unit is smaller than that of the AAO process. The corresponding Shannon index values of the samples collected on 2021.8.20 are H2-AAO (3.596), H2-VFL (4.363), Q2-AAO (3.641), Q2-VFL (4.459), Y2-AAO (3.823) and Y2 -VFL (4.596). The Shannon index of each stage of the VFL device is greater than that of the AAO process. The Simpson index values of the samples collected on 2021.8.12 are: H1-AAO (0.054), H1-VFL (0.331), Q1-AAO (0.051), Q1-VFL (0.286), Y1-AAO (0.055) and Y1-VFL (0.035). In this batch, in contrast to the Shannon index value, the Simpson index of the aerobic and anoxic sections of the VFL unit is larger than that of the AAO process. The corresponding Simpson index values of the samples collected on 2021.8.20 are: H2-AAO (0.094), H2-VFL (0.066), Q2-AAO (0.093), Q2-VFL (0.057), Y2-AAO (0.080) and Y2 -VFL (0.024). The Simpson index of each stage of the VFL device is smaller than that of the AAO process. This indicates that the bacterial richness and diversity of the VFL device are higher than that of the AAO process [25]. In addition, the coverage of all samples is greater than 99%, indicating that the sequencing results can represent the real situation of the samples [26].

Table 6-1. Alpha diversity of microbial community structure.

Time	Sample	Number	OTUs	Shannon	Chao	Ace	Simpson	Coverage
2021.8.12	H1-AAO	56838	847	4.445	955.462	945.732	0.054	0.998
	H1-VFL	40822	700	2.731	847.777	825.112	0.331	0.996
	Q1-AAO	50358	841	4.525	926.150	923.357	0.051	0.997
	Q1-VFL	39878	712	2.950	867.419	843.019	0.286	0.996
	Y1-AAO	46007	899	4.579	972.598	970.218	0.055	0.997
	Y1-VFL	44410	906	4.821	1005.039	972.144	0.035	0.997
2021.8.20	H2-AAO	36331	490	3.596	595.208	572.005	0.094	0.997
	H2-VFL	30360	613	4.363	692.596	663.810	0.066	0.997
	Q2-AAO	39550	510	3.641	633.667	596.686	0.093	0.997
	Q2-VFL	32511	634	4.459	712.981	689.521	0.057	0.997
	Y2-AAO	40033	519	3.823	625.047	591.473	0.080	0.998
	Y2-VFL	37840	574	4.596	663.553	628.744	0.024	0.998

6.3 Analysis of differences in microbial community structure

Figure 6-2 shows the results of metagenomic statistics based on the phylum level. For the 2021.8.12 sample, H1-VFL contains Proteobacteria (77.76%), Bacteroidetes (9.05%), Chloroflexi (6.57%), Acidobacteria (0.42%), Firmicutes (0.60%), Spirochaetes (0.36%), Planctomycetes (0.12%), Parcubacteria (0.22%), unclassified_Bacteria (3.17%); Y1-VFL contains Proteobacteria (56.51%), Bacteroidetes (16.83%), Chloroflexi (5.79%)), Acidobacteria (1.26%), Firmicutes (3.81%), Spirochaetes (2.31%), Planctomycetes (1.25%), Parcubacteria (0.26%), unclassified_Bacteria (8.56%); Q1-VFL contains Proteobacteria (76.46%), Bacteroidetes (9.25%), Chloroflexi (5.65%), Acidobacteria (0.40%), Firmicutes (0.69%), Spirochaetes (0.49%), Planctomycetes (0.13%), Parcubacteria (0.22%), unclassified_Bacteria (4.49%). In the AAO process, H1-AAO contains Proteobacteria (68.06%), Bacteroidetes (8.15%), Chloroflexi (6.43%), Acidobacteria (2.46%), Firmicutes (0.71%), Spirochaetes (1.10%), Planctomycetes (1.73%) %), Parcubacteria (0.99%), unclassified_Bacteria (7.10%); Y1-AAO contains Proteobacteria (64.60%), Bacteroidetes (11.37%), Chloroflexi (6.00%), Acidobacteria (2.06%), Firmicutes (1.97%), Spirochaetes (1.47%), Planctomycetes (1.46%), Parcubacteria (0.44%), unclassified_Bacteria (7.27%); Q1-AAO contains Proteobacteria (66.30%), Bacteroidetes (8.43%), Chloroflexi (6.70%), Acidobacteria (2.11) %), Firmicutes (0.91%), Spirochaetes (1.20%), Planctomycetes (1.63%), Parcubacteria (1.18%), unclassified_Bacteria (7.48%).

It can be seen that Proteobacteria is still the main dominant bacterial phylum, with the most in the aerobic section of the VFL device, reaching 77.76%. Proteobacteria are mostly facultative heterotrophs with both respiration/fermentative metabolism, using organic matter as carbon source, and they are the main participants in COD degradation in sewage treatment systems [27, 28]. There are most microorganisms related to nitrogen cycle in Proteobacteria, which can be divided into five classes: α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, ε-Proteobacteria, and most of them are facultative with respiration/fermentative metabolism [29, 30]. Heterotrophic bacteria, using organic matter as carbon source, are significant players in COD degradation in wastewater treatment systems [31, 32]. Firmicutes are also good at removing COD and degrade organic matter into formic acid, lactic acid, and butyric acid [33, 34]. Bacteroidetes are chemoorganotrophic bacteria, including nitrifying bacteria and denitrifying bacteria, which can degrade many complex organic macromolecular compounds such as proteins and carbohydrates, such as Bacterodia and Flavobacteria [35, 36]. Chloroflexi is strictly anaerobic bacteria that usually degrade polysaccharides, proteins, and organic matter produced by anammox bacteria after aging and play a specific role in forming anammox bacteria [37, 38]. Microbial populations such as Chloroflexi and Acidobacteria are widespread in winter urban sewage treatment systems [39, 40]. Acidobacteria is mostly acidophilic bacteria, which widely exist in various natural environments, play an essential role in ecosystems, and can remove COD [41].

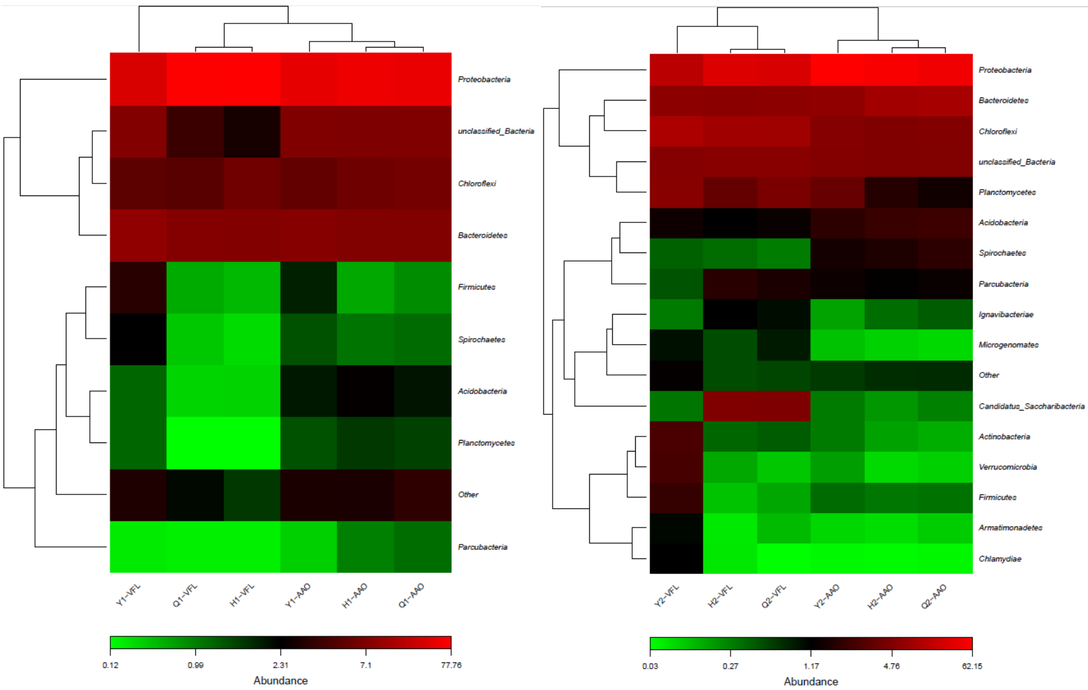

Figure 6-2. Metagenome statistics results are based on phylum level (2021.8.12 sample on the left, 2021.8.20 sample on the right).

Figure 6-3 shows the results of metagenomic statistics based on the family level. The 2021.8.12 sample mainly includes Rhodocyclaceae, Sphingomonadaceae, Comamonadaceae, Anaerolineaceae, Bdellovibrionaceae, Chitinophagaceae, Caldilineaceae, Planctomycetaceae, Porphyromonadaceae, Caulobacteraceae, Geobacteraceae, Bacteroidaceae, Moraxellaceae, Acetobacteraceae, Desulfovibrionaceae, Campylobacteraceae, Spirochaetaceae, Leptospiraceae, Ruminococcaceae, Hyphomicrobiaceae, Aeromonadaceae. The 2021.8.20 sample mainly contains Sphingomonadaceae, Anaerolineaceae, Rhodocyclaceae, Comamonadaceae, Planctomycetaceae, Chitinophagaceae, Rhodospirillaceae Leptospiraceae, Caldilineaceae, Bdellovibrionaceae, Acetobacteraceae, Ignavibacteriaceae, Desulfovibrionaceae, Verrucomicrobiaceae, Saprospiraceae, and Coxiellaceae. In general, the microbial communities of the VFL plant and the AAO process differed at the family level. Sphingomonadaceae detected the most significant number in the AAO process of 2021.8.20 samples, belonging to Proteobacteria, containing glycosphingolipids in the cell membrane, typical aerobic, chemoautotrophic, Gram-negative, rod-shaped, usual colonies in the form of yellow bacteria. Anaerolineaceae belongs to the Chloroflexi phylum and predominates in VFL installations.

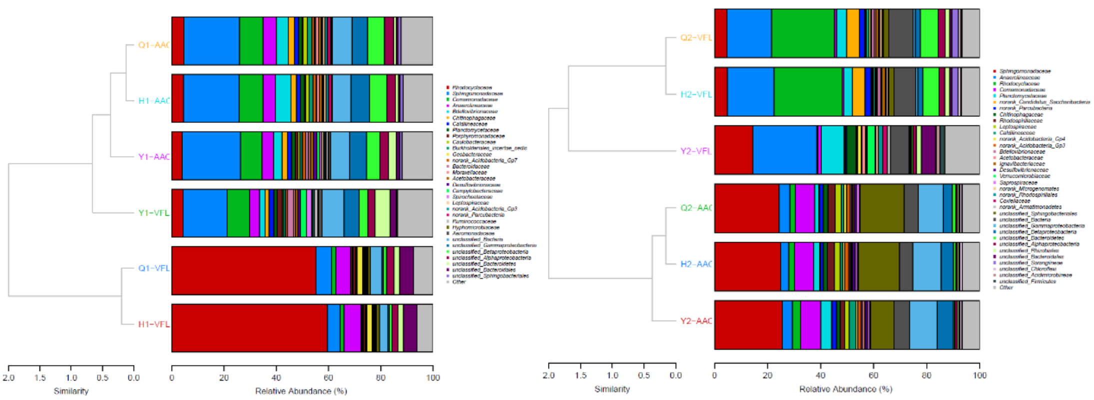

Figure 6-3. Metagenome statistics are based on the family level (2021.8.12 samples on the left, 2021.8.20 samples on the right).

Figure 6-4 shows the results of metagenomic statistics based on the genus level. For the 2021.8.12 sample, H1-VFL mainly contains *Novosphingobium* (3.74%), *Vampirovibrio* (0.12%), *Zoogloea* (0.19%), *Geobacter* (1.95%), *Bacteroides* (0.02%), *Ottowia* (0.06%), *Ferruginibacter* (0.02%), *Aquabacterium* (0.18%), *Desulfovibrio* (0.22%), *unclassified_Rhodocyclaceae* (58.08%), *unclassified_Anaerolineaceae* (4.78%), *unclassified_Bacteroidales* (5.00%); Y1-VFL Mainly include *Novosphingobium* (15.90%), *Vampirovibrio* (1.93%), *Zoogloea* (1.02%), *Geobacter* (0.09%), *Bacteroides* (3.00%), *Ottowia* (0.86%), *Ferruginibacter* (0.74%), *Aquabacterium* (0.48%), *Desulfovibrio* (0.95%), *unclassified_Rhodocyclaceae* (1.71%), *unclassified_Anaerolineaceae* (1.97%), *unclassified_Bacteroidales* (2.32%); Q1-VFL mainly contains *Novosphingobium* (4.76%), *Vampirovibrio* (0.16%), *Zoogloea* (0.14%), *Geobacter* (2.16 %), *Bacteroides* (0.03%), *Ottowia* (0.04%), *Ferruginibacter* (0.02%), *Aquabacterium* (0.20%), *Desulfovibrio* (0.27%), *unclassified_Rhodocyclaceae* (53.77%), *unclassified_Anaerolineaceae* (3.97%), *unclassified_Bacteroidales* (5.28%) .

For the 2021.8.12 sample, and for the AAO sample, H1-AAO mainly contains *Novosphingobium* (20.525%), *Vampirovibrio* (5.20%), *Zoogloea* (1.44%), *Geobacter* (0.06%), *Bacteroides* (0.31%), *Ottowia* (1.11%), *Ferruginibacter* (0.97%), *Aquabacterium* (1.00%), *Desulfovibrio* (0.31%), *unclassified_Rhodocyclaceae* (1.60%), *unclassified_Anaerolineaceae* (2.87%), *unclassified_Bacteroidales* (0.40%); Y1-AAO mainly contains *Novosphingobium* (21.50%) %), *Vampirovibrio* (3.05%), *Zoogloea* (1.07%), *Geobacter* (0.07%), *Bacteroides* (1.15%), *Ottowia* (1.07%), *Ferruginibacter* (1.05%), *Aquabacterium* (0.59%), *Desulfovibrio* (1.29%) , *unclassified_Rhodocyclaceae* (1.55%), *unclassified_Anaerolineaceae* (2.28%), *unclassified_Bacteroidales* (1.15%); Q1-AAO mainly contains *Novosphingobium* (20.45%), *Vampirovibrio* (3.99%), *Zoogloea* (1.52%), *Geobacter* (0.05%), *Bacteroides* (0.37%), *Ottowia* (1.00%), *Ferruginibacter* (1.04%), *Aquabacterium* (1.30%), *Desulfovibrio* (0.30%), *unclassified_Rhodocyclaceae* (1.72%), *unclassified_Anaerolineaceae* (3.38%), *unclassified_Bacteroidales* (0.47%). Compared with the VFL device, *Novosphingobium* has the main advantage in the AAO process [42]. It is Gram-negative bacteria, mostly yellow, obligate aerobic and can produce catalase [43]. Pentose, hexose and disaccharides are converted to acids [44, 45]. In addition, *Vampirovibrio* and *Zoogloea* are the dominant genera in the AAO process. Compared with the samples collected on 2021.8.12, Novosphingobium still has the main advantage in the AAO process in the samples collected on 2021.8.20. *unclassified_Rhodocyclaceae* had a larger proportion in VFL.

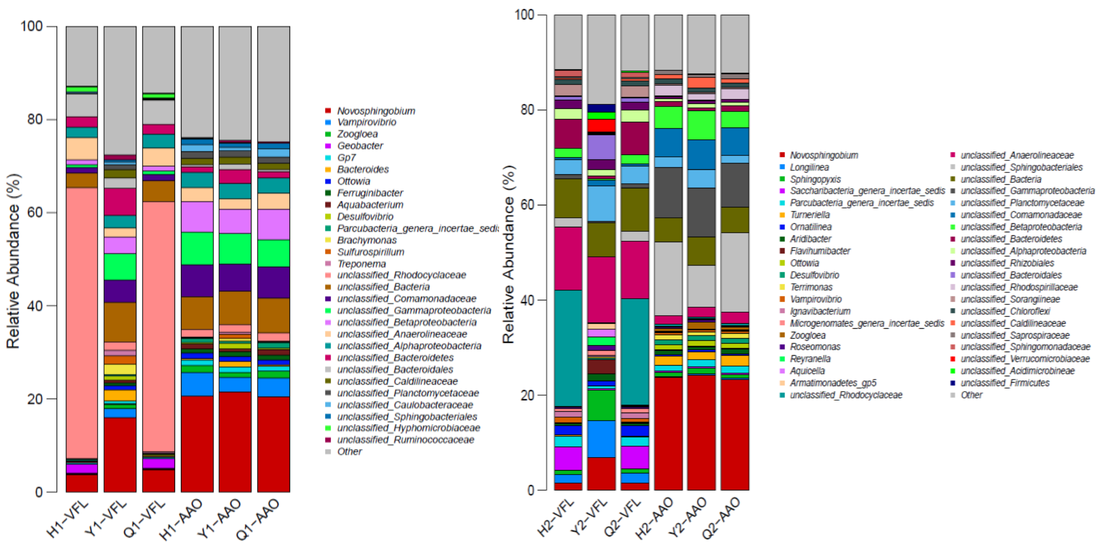

Figure 6-4. Metagenome statistics results based on genus level (2021.8.12 sample on the left, 2021.8.20 sample on the right)

6.4 Gene functions associated with microbial communities

In this experiment, FAPROTAX was used to annotate functional genes in sludge [46]. Figure 6-5 shows the detection results of microbial functional genes in the VFL device and AAO process using metagenomic. Whether it is the sample of 2021.8.12 or the selection of 2021.8.20, these genes are involved in carbohydrate, amino acid, and protein metabolism and are inseparable from the growth and survival of various biological groups in the sludge. As reported, the amino acid metabolism refers to formation and conversion [47, 48]. In a study by Chu et al., they used a two-stage anoxic-oxic (A/O) system to treat wastewater, results of metagenomic sequencing showed that the relative abundance of amino acid metabolism and carbohydrate metabolism in the primary AO process were both higher than that in the secondary AO process [49]. Studies have also shown that functional genes involved in the metabolism of phosphorus, sulfur, and aromatic compounds are the guarantees for the high performance of sludge [50]. Among them, it is worth noting that anammox is an essential process of the global nitrogen cycle in which microorganisms are involved. The bacteria-mediated process caused a great stir in the scientific community at the beginning of its discovery. Through the anaerobic ammonium oxidation process, nitrite and ammonium can be directly converted into nitrogen, removing ammonia nitrogen in wastewater [51]. In the 2021.8.12 sample, anammox function genes were also detected. In addition, genes involved in nitrification, denitrification and nitrogen fixation were detected in the 2021.8.12 samples and the 2021.8.20 samples.

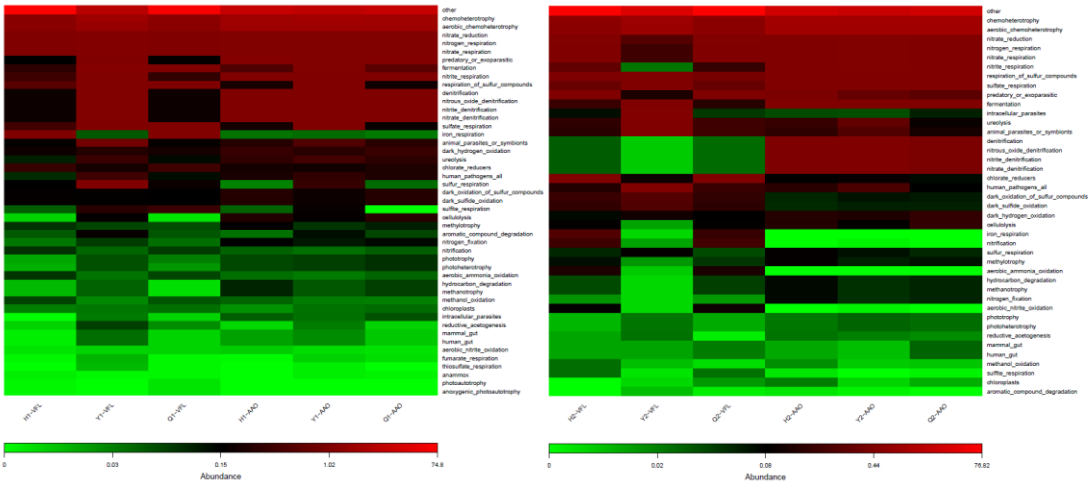

Figure 6-5. Microbial functional gene detection in VFL device and AAO process with the help of metagenomic (2021.8.12 sample on the left, 2021.8.20 sample on the right)

6.5 Summary

The Simpson index of each stage of the VFL device is smaller than that of the AAO process. This indicates that the bacterial richness and diversity of the VFL device are higher than that of the AAO process. In addition, the Coverage of all samples is greater than 99%, indicating that the sequencing results can represent the real situation of the samples. Proteobacteria was the main dominant bacterial phylum, which was the most in the aerobic section of the VFL device, reaching 77.76%. The microbial communities of the VFL plant and the AAO process were different at the family level. Sphingomonadaceae detected the most significant number in the AAO process of 2021.8.20 samples, belonging to Proteobacteria, containing glycosphingolipids in the cell membrane, typical aerobic, chemoautotrophic, Gram-negative, rod-shaped, usual colonies in the form of yellow bacteria. Anaerolineaceae belongs to the Chloroflexi phylum and predominates in VFL installations. *Novosphingobium* has a significant advantage in the AAO process. *unclassified_Rhodocyclaceae* had a larger proportion in VFL. In the 2021.8.12 sample, anammox function genes were detected. In addition, genes involved in nitrification, denitrification and nitrogen fixation were detected in the 2021.8.12 samples and the 2021.8.20 samples.

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Chapter 7

RESEARCH ON SMALL SEWAGE TREATMENT TECHNOLOGY

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With society and economic development, China faces water shortage problems, a low utilization rate of water resources, unbalanced development and utilization [1], excessive exploitation of groundwater, and a low utilization rate of water resources [2]. According to the relevant annual environmental statistics report, domestic sewage discharged from cities and towns in China was 48.51 billion tons in 2015， and most villages and towns lacked sewage collection and treatment facilities. Based on the actual situation, the corresponding countermeasures are put forward, such as strengthening infrastructure construction, developing sewage treatment technology, and improving the water resources recycling utilization rate [3].

The sewage is diverse and complex in Jilin Province. The water quality and quantity are unstable in cold winter so the conventional treatment fails to meet discharge standards [4]. Jilin Province is economically underdeveloped. Therefore, economic factors should also be considered while considering sewage treatment technology. How to treat village sewage with unstable water quality and quantity in winter is an urgent and realistic problem to be solved.

Compared with the high investment, complex operation, and different water quality characteristics of sewage treatment in big cities, the village sewage treatment has just started in Jilin Province. These technologies can be used for reference but not copied. The AAO process, SBR, and MBR are commonly used in village sewage treatment in Jilin Province [5].

7.1 Rural Sewage Treatment Technology in Jilin Province

The core of each sewage treatment is the process closely related to influent water quality, effluent requirements, treatment capacity, investment size, and other factors [6]. Because the primary pollutant of municipal wastewater is organic matter, most municipal wastewater is treated by biological treatment technology at home and abroad, which can be divided into activated sludge and biofilm methods [7]. Activated sludge wastewater treatment plants account for the vast majority of biofilm treatment's low efficiency and poor sanitary conditions [8].

There are three types of processes which widely used in Jilin Province: (1) Traditional AAO process, (2) SBR process, and (3) MBR process. This paper will briefly introduce the three wastewater treatment processes and a new improved AAO treatment technology (VFL).

7.1.1 Traditional AAO process

The traditional activated sludge process is the earliest sewage treatment process in the world. It has high efficiency in removing organic matter and suspended matter. For urban sewage, BOD_5 and SS of effluent should be below 30mg/L. The traditional AAO process has the effect of nitrogen and phosphorus removal simultaneously [9]. The principle is that phosphorus is released in the anaerobic zone and absorbed in the aerobic zone to achieve phosphorus removal [10]. Pollutants are oxidized and degraded in the aerobic zone to remove COD and BOD_5. At the same time, under the action of nitrifying bacteria, ammonia nitrogen converted by organic nitrogen continues to be converted into nitrite nitrogen and nitrate nitrogen, and a large amount of mixed liquor with nitric acid nitrogen was refluxed to an anoxic zone for denitrification [11]. The flow chart is shown in Fig. 7-1.

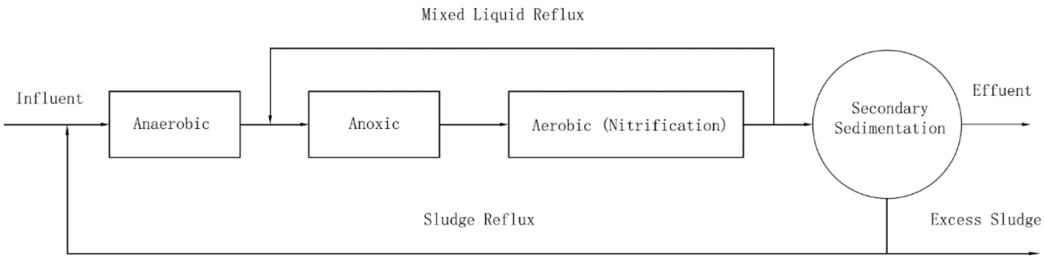

Figure 7-1. AAO process flow chart.

The main advantages of this process are the high removal rate of COD, BOD_5, and SS, the high removal efficiency of nitrogen and phosphorus, low operation cost, less land occupation and good effluent quality. The disadvantages are high requirements for operation management and increased investment [12]. For the small-scale processing program in Jilin Province, the advantages and disadvantages of AAO are summarized as follows:

Advantages:

1. Microorganisms with different populations and different environmental conditions cooperate (anoxic, anaerobic, and aerobic) to remove organic matter and remove nitrogen and phosphorus simultaneously [13].
2. The process is simple, with short cycle period and high treatment efficiency.
3. Filamentous bacteria do not multiply in large quantities under the alternating operation of three oxygen environments. SVI is generally less than 100 and sludge bulking does not occur.
4. Sludge sedimentation is good.

Disadvantages:

1. The phosphorus content in sludge is high, generally more than 2.5%, so phosphorus removal is mainly achieved through sludge discharge.
2. The effect of denitrification is restricted.
3. Dissolved oxygen is strictly controlled.
4. The effect of nitrogen removal in winter is not good.
5. Small-scale equipment cannot satisfy sludge discharge and storage.
6. Poor impact resistance.

7.1.2 Traditional and Improved SBR process

7.1.2.1 Traditional SBR process

SBR process is also known as the sequencing batch activated sludge process [14]. The intermittent operation distinctively features it, periodic influent, and effluent after aeration and precipitation for cyclic processing. The process flow chart is shown in Fig. 7-2. The core technology of SBR is the main reaction tank, which integrates primary sedimentation tank, biochemical tank and sedimentation tank, without sludge reflux. The process has been adopted by Crundy (3000m^3/d) wastewater treatment plant in Australia in the United States and Tamwpfth wastewater treatment plant.

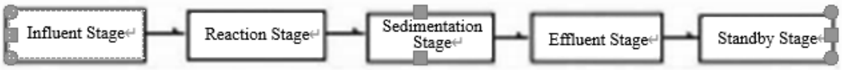

Figure 7-2. SBR process flow chart

The process has the following advantages: stable and good effluent quality, simple operation and management, high level of automatic control, small area, low cost, and flexible operation. However, the shortcomings, such as blockage of aeration system, high failure rate, and high requirement of monitoring means, also affect the use of SBR in winter [15].

7.1.2.2 CAST process

CAST is an improved SBR process, with the best nitrogen and phosphorus removal efficiency among all the improved SBR processes [16]. The most significant improvement of this process to SBR is the selective zone at the front of the reactor. The sewage first enters the selective zone, and mixes with the reflux mixture of the autonomous reaction zone. Under anaerobic conditions, the selective zone is equivalent to a pre-anaerobic tank, increasing the phosphorus removal efficiency [10]. Another characteristic of this process is that it uses the principle of simultaneous nitrification and denitrification to denitrify [17, 18]. In the main reaction zone, the dissolved oxygen is controlled to no more than 15 mg/L in the early stage of the reaction period which is in anoxic state. The original nitrate nitrogen and the nitrate nitrogen produced by simultaneous nitrification are used for denitrification. At the later stage of the reaction, the oxygen supply is increased to an aerobic state, so as to complete the phosphorus removal reaction, and maintain sufficient dissolved oxygen in effluent. Due to simple design and operation management, this process has been applied to sewage treatment by many small sewage plants in Jilin province.

However, shortcomings are also obvious, such as higher requirements for self-control, high power consumption, low volume utilization rate, sludge stability inferior to anaerobic nitrification, and poor low-temperature adaptation.

7.1.2.3 MSBR process

MSBR is a modified SBR in combination of AAO and SBR processes, incorporating advantages of the both. Therefore, the effluent is stable [19]. MSBR is a continuous and efficient sewage treatment process [20], for which automatic computer control is available due to easy operation, small volume and single tank. It can effectively treat wastewater containing high concentrations of organic matter, nitrogen and phosphorus [21]. However, MSBR process needs further optimization and improvement due to the complex structure, varied equipment, high cost and inconvenient management [22].

7.1.3 MBR process

MBR (Membrane Bio-Reactor) is a water treatment technology that uses membrane separation process to replace secondary sedimentation tank in traditional activated sludge process [23, 24]. There are many kinds of membranes, which are classified into reaction membranes, ion exchange membranes and osmotic membranes by separation mechanism.

MBR uses membrane separation equipment to intercept activated sludge and macromolecule pollutants in biochemical reaction tank, omitting the secondary sedimentation tank [25]. In traditional wastewater biological treatment technology, sludge-water separation is accomplished by gravity in secondary sedimentation tank. Its separation efficiency depends on the sedimentation performance of activated sludge. The sludge-water separation efficiency is higher when the sedimentation is better. While, the sludge sedimentation depends on the operating condition of aeration tank. To improve the sedimentation performance, the operating conditions of aeration tank must be strictly controlled, limiting the application scope of this method. Membrane-bioreactor technology significantly strengthens the function of bioreactor through membrane separation technology, which greatly increases the concentration of activated sludge. Given interdependence of hydraulic retention time (HRT) and sludge age (SRT), MBR achieves the separation of sludge retention time and hydraulic retention time, which greatly improves the efficiency of solid-liquid separation. The increase of activated sludge concentration in the aeration tank and the emergence of specific bacteria (dominant microbial population) in the sludge also accelerate the biochemical reaction. The flow chart is shown in Fig. 7-3.

At the same time, there are some shortcomings in membrane-bioreactor, mainly in the following respects: (1) high cost of the membrane, which makes the higher investment of membrane-bioreactor infrastructure than that of traditional sewage treatment process; (2) membrane prone to fouling, which brings inconvenience to operation and management; (3) high energy consumption: firstly, the sludge-water separation process of MBR must maintain a specific membrane driving pressure; secondly, to maintain sufficient oxygen transfer rate, aeration intensity must be increased in view of very high concentration of MLSS in MBR pool; additionally, to increase membrane flux and reduce membrane fouling, flow rate must be increased to scour the membrane surface, resulting a higher energy consumption than traditional biological treatment process.

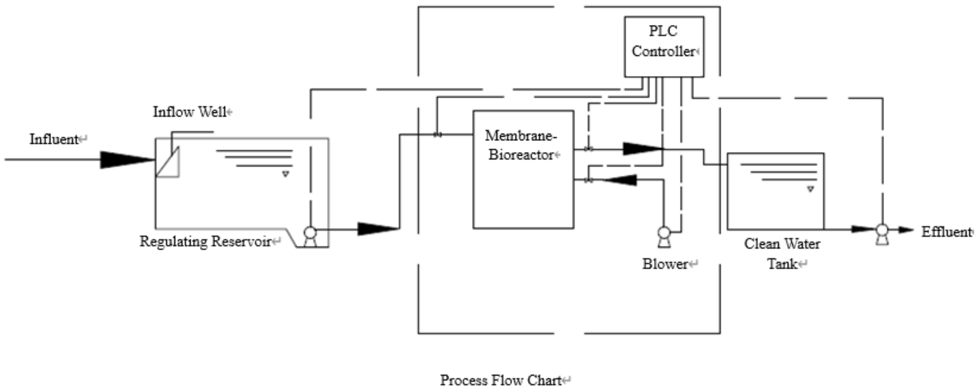

Figure 7-3. MBR process flow chart

7.1.4 VFL Process

VFL (Vertical Flow Labyrinth) technology originated in Slovakia in 1990. In 1992, it was used to construct the first small sewage treatment station. Mr. JURAJ, the inventor of VFL technology, initially used it to treat domestic sewage in single dwelling houses, adapting to the domestic drainage featured by stable and qualified effluent at a significant fluctuation of discharge. At the same time, the treatment equipment should meet the requirements of EU countries for sludge discharge. (For example, small sewage treatment plants can only discharge sludge once a year in France, and twice a year in Germany). Through 30 years of development, the technology has been widely applied in more than 35 countries worldwide. The size of treatment ranges from 0.9T/d per household to 50000T/d for small municipal wastewater, in the form of underground integrated equipment to large reinforced concrete composite pools. This technology adapts to the characteristics of scattered households and point drainage in Europe, i.e., dispersed sewage discharge and great fluctuation. It technically solves the problem of impact resistance and stable operation of small sewage treatment facilities, and can be fully automated and unattended. The technical characteristics of VFL determine that it is the best solution for domestic sewage treatment in the process of urbanization and new rural construction in China.

VFL process adopts vertical flow labyrinth structure in anaerobic and anoxic zones, which greatly extends the flow of anaerobic and anoxic zones in structure, eliminating the adverse effects of reflux activated sludge on anaerobic and anoxic zones, and dramatically improves its nitrogen removal efficiency. It is also helpful for phosphorus removal, control and adaptation of carbon sources utilization in anaerobic and anoxic zones. The process flow chart is shown in Fig. 7-4.

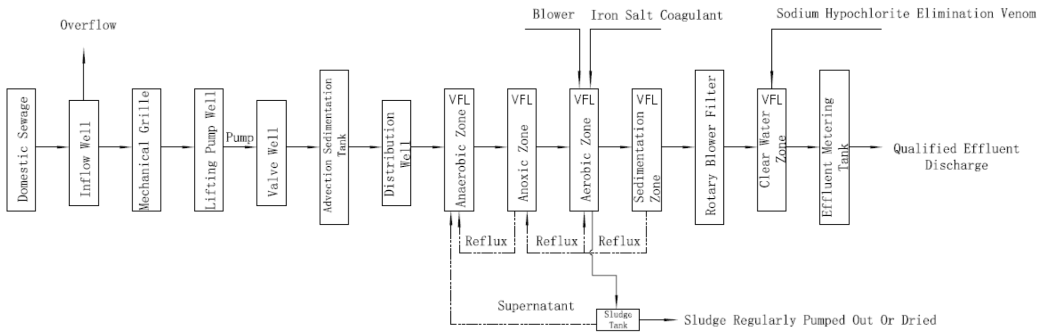

Figure 7-4. VFL process flow chart.

This process not only has good effect on nitrogen and phosphorus removal, but also has the flexibility of adjusting operating parameters to meet the changes in water quality and strong anti-impact load capacity. It is also prospective in coping with improving effluent standards and the limitation of sludge discharge.

The effluent from the VFL process precipitation tank enters the rotary drum filter, which has strong impact resistance, good filtration effect, small head loss and all-day automatic operation. It is a sewage treatment equipment matching to the VFL process, ensuring the qualified effluent. The system is equipped with mixed liquor and sludge reflux different from the traditional AAO process. The reaction process is shown in Fig. 7-5.

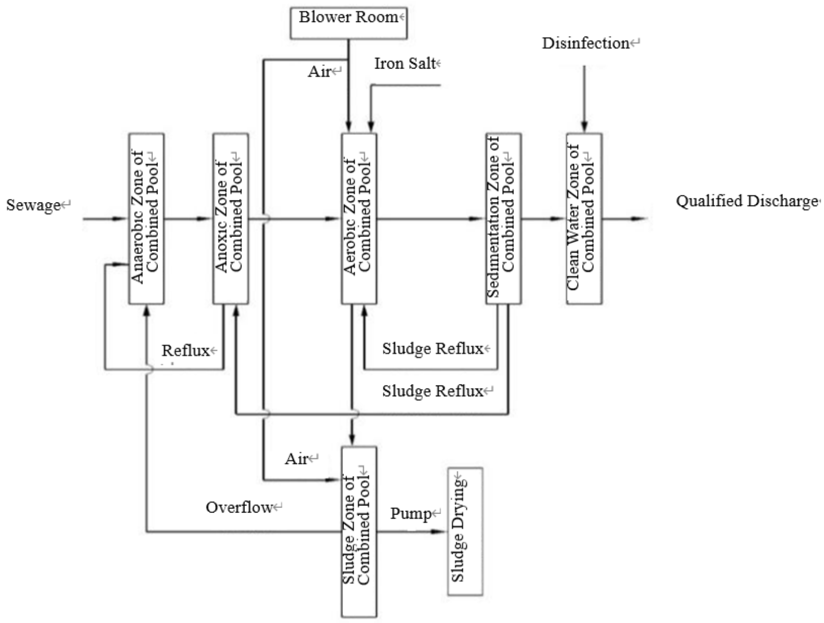

Figure 7-5. VFL Reaction Flow Chart.

A part of activated sludge in sludge bucket of sedimentation zone returns to the front section of anoxic zone, which contains dissolved oxygen. Similarly, due to the characteristics of vertical flow structure, water flows to the second and third compartments of anoxic zone. The dissolved oxygen concentration decreases rapidly, allowing the thorough denitrification in a longer anoxic process. Moreover, making fully use of carbon source (BOD_5) in sewage, the denitrification rate is much higher than that of denitrification by endogenous respiration. Therefore, it requires only a short denitrification residence time and small volume.

The VFL process has a unique sludge recycling route. In addition to the half of the activated sludge used for denitrification, the other half returns to the aerobic zone. The mixed liquid in the aerobic zone is discharged into the sludge zone, and then returned to the anaerobic zone. This flow allows sludge to remain active. The microorganism is in a low-activity state in the aerobic zone, basically a low-load complete mixed reaction zone. After entering the sludge pool, the microorganism gradually becomes the endogenous respiratory state, where the sludge is partially digested. The remaining naturally screened microorganisms then enter the high-load anaerobic zone, re-activating their activity. The activity of adsorbing and degrading organic substances is gradually enhanced, which improves the reaction efficiency and treatment effect. At the same time, sludge can remain active for a long time and realize sludge reduction in self-metabolism.

A new and efficient biological treatment device based on VFL technology combines anaerobic, anoxic and aerobic to create the most favorable physiological environment for the microorganisms needed for sewage treatment, fully realizes the functions of organic matter degradation, nitrification and denitrification, biological phosphorus accumulation, and determines the effective volume of the structure areas according to the water quality of influent and effluent water. Aeration and sludge reflux are controlled by monitoring redox potential in aerobic zone, and the whole system is managed accordingly.

The VFL reactor is symmetrical in left and right, with the middle long and narrow part as sludge tank. Starting unilaterally or bilaterally is decided depending on water quantity. Both anaerobic and anoxic zones of the reactor are vertical flow maze structures. The reactor is divided by its built-in vertical baffle into several reaction chambers in series, each of which is a relatively independent upflow and downflow sludge bed system, where the sludge exists in the form of granulation or flocculation. The water flow is guided by the baffle to flow upward and downward, passing the sludge bed in the reaction chambers one by one. Substrates in the influent are degraded and removed by fully contacting the microorganisms. The front section of the reactor is anaerobic zone. Sewage enters anaerobic zone, anoxic zone, aerobic zone and sedimentation zone successively along the baffle.

7.1.4.1 VFL process characteristics

(1) Strong shock load resistance

VFL is optimized in term of pool structure and operation management. The whole system operates at high sludge concentration (sludge concentration 7-8g/L in anaerobic and anoxic zones, 3-4g/L in aerobic zone), with full capacity against impact load.

(2) Good and stable effluent quality

VFL technology can carry out biochemical reactions thoroughly without any advanced treatment (after biochemical reactions only through precipitation clarification and filtration), and maintain stability to meet the first A standard of "Discharge standard of pollutants for municipal wastewater treatment plant" (GB18918-2002), or even better water quality. Therefore, advanced treatment equipment and machine room are not needed.

(3) Low sludge production

VFL technology considers both sludge reduction and harmlessness while treating sewage, with a very low sludge yield. The sewage treatment plant constructed by VFL technology may discharge sludge for the first time after normal operation of 2-3 years, and then every three months.

(4) Adaptive to stringent environmental requirements

By optimizing the operation management of system, VFL technology eliminates the odor of whole system, including anaerobic zone and sludge zone. This process gets to the root of odor in common treatment process which obsesses most of wastewater treatment plants. Therefore, without any deodorization device, the wastewater treatment station is suitable for any area even with strict environmental requirements.

(5) Simple system, low energy consumption, and small daily maintenance workload

The types and quantities of VFL technology equipment have been reduced to the minimum. Except the lifting equipment and sand removal facilities at the sewage inlet, biochemical combined pool is the core, supported by only blower and dispensing device. In this way, the daily maintenance workload of equipment has been greatly reduced, saving the energy consumption to the greatest extent.

(6) No further investment

There is no filler in the combined pool, avoiding blocking problems, and requiring no regular replacement and cleaning. The long-term use of contact oxidation process and biological filter process fillers will cause blockage, and the material will also appear aging and falling off after 5 years, so it must be replaced regularly. At present, MBR process membrane components generally have a service life of 3-5 years. Using domestic membrane, the total investment of equipment is about 40% higher than that of ordinary process, and the imported membrane is 80% higher [5]. Moreover, the daily maintenance of backwashing and chemical cleaning requires supporting machine rooms and equipment, introducing large workload and higher requirements for managers.

7.2 Practical cases in Jilin province

Mingcheng Economic Development Sewage Treatment Station is located in Panshi City, Jilin Province, with a designed capacity of 2500 tons per day. The main sewage collected by the Development Zone sewage treatment plant comes from old town, new district in the east city, and hardware industrial park. Old town and new district in east city are commercial and living areas. The main industry of hardware industrial park is building materials processing, which relying on iron and steel industrial park. The sewage discharge from building materials processing industry is less, accounting for only about 12% of the total sewage.

According to the actual situation of the town and considering the development trend of Mingcheng Economic Development Zone, the designed water quality of the sewage treatment plant of this project is determined as shown in Table 7-1.

Table 7-1. Designed water quality of Mingcheng sewage treatment plant.

Index	COD	BOD_5	SS	TN	NH_3-N	TP
Date	250	120	200	40	25	5

After a long period of operation, the VFL process operates well in Mingcheng Sewage Treatment Plant. In the coldest winter, the effluent can meet the Grade 1 Class A standard of "Discharge standard of pollutants for municipal wastewater treatment plant" (GB18918-2002). The winter operation data are shown in Fig. 7-6.

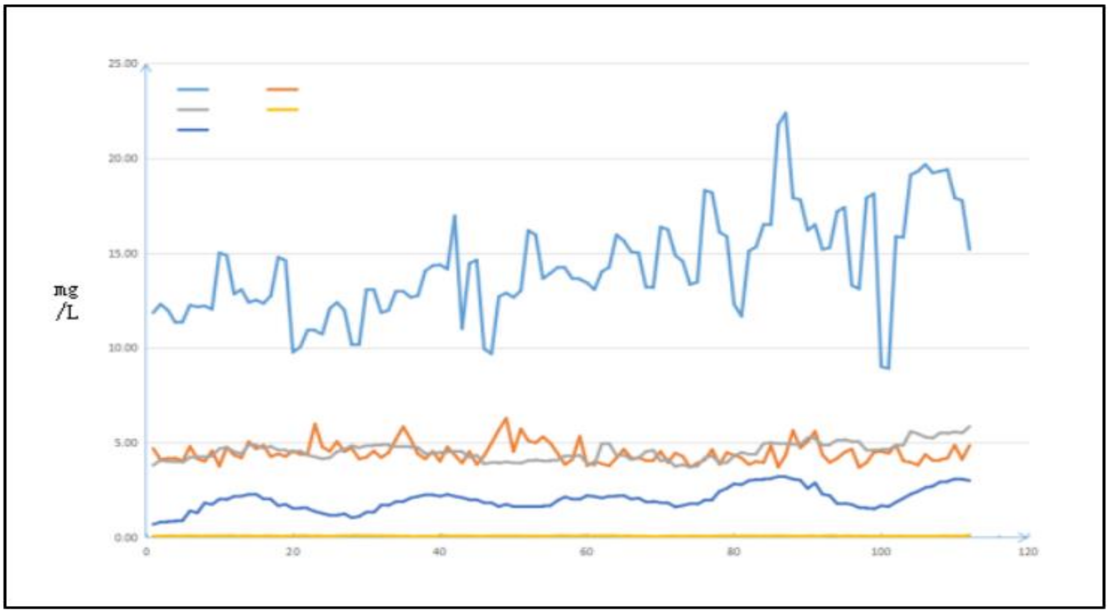

Figure 7-6. Winter Operation Data of Mingcheng Sewage Treatment Plant

7.3 Conclusion

The excellent operation of Mingcheng Sewage Treatment Plant shows that, VFL process can meet the requirements of small-scale sewage treatment equipment in Jilin Province for resisting the fluctuation of water quality and quantity with a good wintering ability, very low sludge yield and low operation cost. Therefore, the VFL process should be preferred in the selection of small sewage treatment equipment in Jilin Province.

Compared with the traditional AAO, SBR and MBR processes, VFL process shows the following superiorities:

1. This process possesses a strong shock load resistance, and it can cope with fluctuations in water quality and quantity.

2. Stable effluent.

3. Low sludge production. It can significantly save the sludge quantitation cost.

4. Simple system and small daily maintenance workload significantly reduce the operating costs.

5. Adapted to cold water intake in winter in Northern China.

6. The system only occupies a small area, significantly lowering the cost.

7. Good sludge quality and strong processing capacity under VFL hydraulic conditions.

The operations of sewage treatment facilities in Mingcheng in summer and winter showed that the VFL process could satisfy Jilin Province's requirements for small-scale sewage treatment equipment. It can withstand fluctuations in water quality and quantity, and possesses strong resistance to cold. Besides, it has an extremely low sludge yield and a low operating cost. Hence, the VFL process shall be the priority selection for small-scale sewage treatment equipment in Jilin Province.

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Chapter 8

SELECTION ANALYSIS OF VFL AS A SEWAGE TREATMENT TECHNOLOGY IN GREEN BUILDINGS

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CHAPTER 8: SELECTION ANALYSIS OF VFL AS A SEWAGE TREATMENT TECHNOLOGY IN GREEN BUILDINGS

8.1 Green building

As a pillar industry supporting China's national economy, the construction industry is growing rapidly with high resource consumption and environmental impact [1, 2]. To a large extent, buildings have an impact on global climate and environmental issues [3], and the development of green buildings to form a modern construction in harmony with man and nature has become a favorable option. It is well known that the development of green building can not only improve people's living environment and boost people's livelihood, but also is the strategic direction of national development in the new era and the way to implement the construction of ecological civilization [4, 5].

Green building is a high-quality building that saves resources, protects the environment, reduces pollution, and provides people with a healthy, suitable, and efficient space for use during its whole life cycle, maximizing the harmonious coexistence between humans and nature [6, 7]. Green building can effectively integrate resources and environmental issues, to solve the problem of harmonious development of human beings and the environment to the greatest extent, which also plays an important role in sustainable development. There is a complete set of system indicators for the green building. Green buildings should be evaluated and analyzed from multiple perspectives such as evaluation system, operational performance, building benefits and environment. The index system is shown in Fig. 8-1.

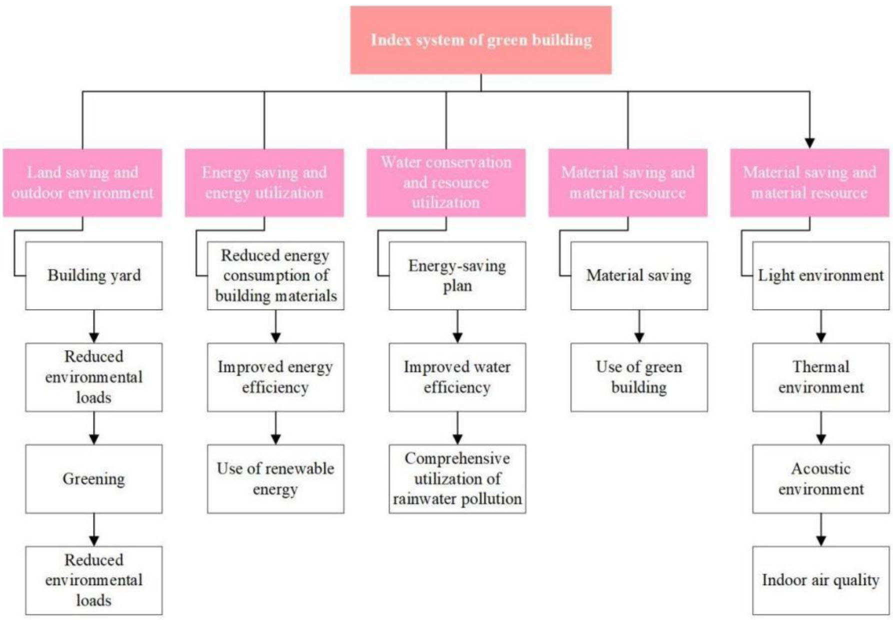

Figure 8-1. Index System of Green Building.

The concept of ecological architecture was first proposed by Italian designers in the 1960s, and the technology, equipment and renewable energy technologies for green buildings gradually matured until the end of the 20th century [8]. There is a lot of development of green building in various countries and focus on different project facilities. For example, solar photovoltaic systems, low carbon emission technologies, glass technologies, ground source heat pump cooling and daylighting technologies in the UK have achieved significant results in the area of sustainable buildings; Germany focuses on low environmental impact infrastructure such as porous permeable pavements, planted roofs and open gardens in its infrastructure; the application of solar, hydro and wind energy as the basis for energy production in Sweden provides sustainable energy for green buildings; in addition, some integrated projects have been designed in Europe with the main aim of reducing the thermal and electrical energy consumption [9]. The above initiatives show that the system of green buildings is also evolving and that such buildings are more friendly to the environment.

8.2 Water resources utilization in green buildings

As we all know, water resources are the valuable wealth of human beings, and there are currently widespread problems such as water shortage and great difficulties in sewage treatment. Sewage treatment has become an urgent problem worldwide [10]. The sewage treatment process also creates problems such as high carbon footprint, secondary pollution and an energy consumption-based treatment model. Therefore, the water resources utilization in green buildings should not only save water and decarbonize sewage treatment facilities, but also recycle and reuse water resources.

Currently, both domestic and international studies are being conducted on the regeneration and reuse of water resources. The water resources utilization in green buildings is mainly for reclaimed water treatment and reuse and rainwater utilization.

(1) Reclaimed water treatment and reuse

Reclaimed water reuse is a water supply system in which all kinds of drainage water, such as domestic drainage water, cooling water and rainwater, are used back in the building or residential area as miscellaneous water after proper treatment in a civil building or residential area [11]. Reclaimed water reuse can not only effectively use and save the limited and valuable fresh water resources, but also reduce the sewage and wastewater discharge, minimize the pollution of water environment, and also alleviate the overload of urban sewers [12].

(2) Rainwater utilization

At present, the main techniques of rainwater utilization in green buildings at home and abroad are in situ infiltration, storage infiltration, and rainwater storage using artificial or natural water bodies [9, 13]. The collection and reuse of rainwater solves the short-term problem of water resources to a large extent.

There are two main ways in which rainwater is collected in practical applications: ground collection and roof collection. The collected rainwater sinks into a dedicated rainwater collection line and is transported to the treatment center. Depending on the collection location, the collected rainwater can be divided into the following categories.

① Roof rainwater, with less pollutants and high water collection efficiency, is the primary choice for collection and reuse, see Fig. 8-2 for the specific process.

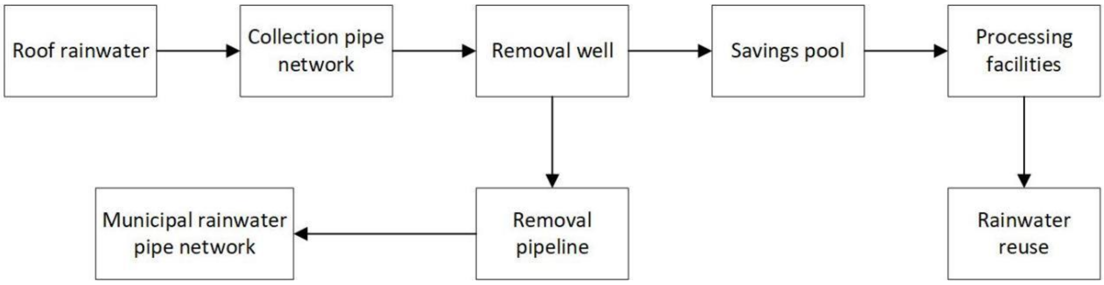

Figure 8-2. Flow of Roof Rainwater Collection.

② Outdoor roads, especially motor vehicle pavement with severe pollution, should not be collected for reuse, the specific process is shown in Fig. 8-3.

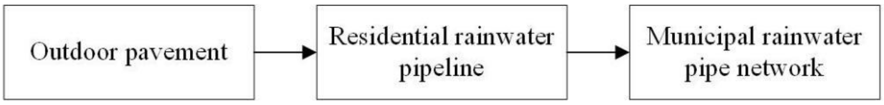

Figure 8-3. Flow of Outdoor Road Rainwater Collection.

③ Outdoor green space, with very clean rainwater, but the collection efficiency is low and uneconomical, see Fig. 8-4 for the specific process.

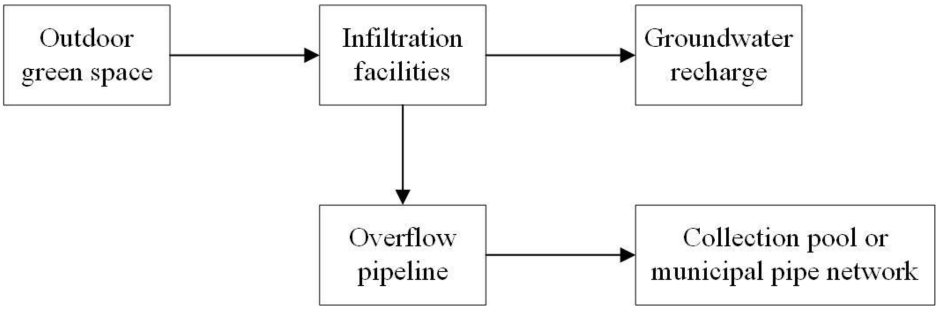

Figure 8-4. Flow of Outdoor Green Space Rainwater Collection.

In the process of water resources reclamation and reuse, in addition to reclaimed water reuse and rainwater utilization, it is also necessary to select the appropriate treatment technology to efficiently remove pollutants from wastewater, so as to achieve the energy saving and emission reduction [14]. The proper treatment technology not only reintegrates water resources, but also maximizes the effectiveness of water resources for the same amount of time and money.

8.3 Analysis of the VFL as a sewage treatment technology

VFL is a new type of biological treatment device. The VFL biochemical tank is equipped with vertical deflectors in the anaerobic and anoxic zones, separating the anaerobic and anoxic zones into tandem reaction chambers, each of which is a relatively independent up-and-down flow sludge bed system that uses excellent internal hydraulic flow patterns to achieve enhanced system biosolids retention capacity with extended flow paths for ultimate biodegradation of pollutants. Therefore, VFL has high efficiency of pollutant removal and small footprint, which is one of the criteria to meet the evaluation of green building.

8.3.1 Analysis of the removal rate of VFL and AAO treatment of sand washing wastewater in Huzhou

On the basis of the climatic conditions at the experimental site, this paper divided the experimental data into five phases as sampled at Huzhou, namely 2021.08.01-2021.08.31 (Phase I), 2021.09.01-2021.09.30 (Phase II), 2021.10.01-2021.10.31 (Phase III), 2021.11.01 -2021.11.30 (Phase IV) and 2021.12.01-2021.12.29 (Phase V), with the average temperatures of 34 ℃, 29 ℃, 23 ℃, 17 ℃ and 11 ℃ for the five phases, respectively.

Table 8-1. Comparison of Removal Rates of VFL and AAO at Different Phases.

		Influent water (mg/L)	VFL effluent water (mg/L)	AAO effluent water (mg/L)	VFL removal rate (%)	AAO removal rate (%)
COD	Phase I	500.0	125.0	128.9	75.0	74.1
	Phase II	477.6	124.0	137.6	74.0	71.2
	Phase III	530.1	139.4	157.7	74.1	70.3
	Phase IV	507.0	153.7	152.3	69.7	69.9
	Phase V	493.5	157.9	157.3	68.0	68.1
BOD_5	Phase I	289.9	31.5	34.0	89.2	88.2
	Phase II	291.7	36.0	39.3	87.7	86.5
	Phase III	287.6	32.9	37.3	88.6	87.1
	Phase IV	294.4	42.8	44	85.4	85.0
	Phase V	290.1	56.5	66.0	80.4	77.0
TN	Phase I	35.7	6.8	7.1	80.9	79.8
	Phase II	33.6	5.82	5.9	82.1	81.8
	Phase III	32.8	5.9	6.1	81.8	81.1
	Phase IV	31.3	6.6	7.0	78.5	77.0
	Phase V	24.5	6.3	6.44	75.3	73.6
NH_4^+-N	Phase I	31.3	3.3	3.8	89.5	87.6
	Phase II	29.0	3.0	3.3	89.7	88.8
	Phase III	29.0	3.2	3.7	90.2	88.5
	Phase IV	27.1	3.6	4.4	86.2	83.3
	Phase V	21.1	4.0	4.4	81.6	79.1
TP	Phase I	2.3	0.6	0.6	72.7	73.4
	Phase II	2.43	0.72	0.7	70.2	70.7
	Phase III	2.5	0.8	0.8	69.4	67.9
	Phase IV	2.5	0.8	0.9	67.0	62.3
	Phase V	2.1	0.8	0.84	61.0	60.4

Table 1.1 shows a comparison of the removal rates of pollutants from wastewater at different phases for VFL and AAO, with the same concentrations of each pollutant in both VFL and AAO influent. During the operation, the average influent COD concentrations at different phases were

500.0, 477.6, 530.1, 507.0 and 493.5 mg/L. The removal of COD by both VFL and AAO showed a decreasing trend with decreasing temperature, therefore, the temperature was the main influencing factor on the bioreactor. The mean influent COD concentrations were similar in phases I and V, but the removal of COD by VFL and AAO was 75.0%, 74.1% (Phase I) and 69.7%, 69.0% (Phase V) in these two phases, respectively, which indicates that low temperature leads to microorganisms with lower metabolic activity and also inhibits intracellular enzyme activity [15]. It is obvious that the removal efficiency of VFL for COD is higher than that of AAO, and the performance of VFL for pollutant removal is more stable as the temperature decreases. When the average temperature decreases from 34 ℃ to 23 ℃, the variation of VFL for COD removal ranges from 74.0% to 75.0%, and the removal rate of AAO for COD decreases from 74.1% to 70.3%.

As can be seen from Table 8-1, the variation of influent pollutant concentrations at different phases is relatively small, which indicates that the water quality of sand washing wastewater is relatively stable. The removal efficiency of both VFL and AAO for COD, BOD_5, TN, NH_4^+-N and TP decreased gradually with decreasing temperature, and VFL was less affected by temperature and the concentration of pollutants in the effluent water was lower than that of AAO. Meanwhile, the effluent water concentration of VFL is generally lower than that of AAO even at the same temperature and influent concentration, which indicates that VFL has a higher removal efficiency for pollutants compared to AAO. In addition, the hydraulic retention time of VFL is smaller than that of AAO, and AAO requires a separate sedimentation tank with a larger footprint, which is another way to verify that VFL is more suitable as a sewage treatment equipment for green buildings compared to AAO.

8.3.2 Comparative analysis of the environmental benefits of VFL and AAO treatment of sand washing wastewater in Huzhou

Unlike other projects, sewage treatment projects belong to environmental protection projects, with the main goal of improving environmental pollution problems to bring economic and environmental benefits [16, 17]. Environmental benefits refer to the benefits and results brought to the natural ecosystem by the production activities in the process of human economic development [18]. COD content and ammonia nitrogen content are the two indicators that sewage treatment plants focus on controlling [19]. Therefore, the environmental benefits come mainly from the reduction of these two types of substances.

The environmental benefits in this paper refer to the environmental benefits resulting from the reduction of COD and ammonia nitrogen by VFL and AAO in the treatment of sand washing wastewater, so the environmental benefits can be expressed by the following equation.

$$\mathrm{Es} = \mathrm{ECOD} + \mathrm{ENH_4^+\text{-}N} \quad (1\text{-}1)$$

Environmental benefits differ from economic benefits and therefore the cost reductions cannot be calculated in the form of monetary measures [20], but environmental benefits can be expressed through indirect calculation methods. The environmental benefits from COD reduction [21] are shown in Equation 1-2:

$$\mathrm{ECOD} = \mathrm{QCOD} \times 365 \times 0.25 \times \mathrm{P_{coal}} \qquad (1\text{-}2)$$

Where ECOD is the environmental benefit of COD (RMB¥/yr); *QCOD* is the amount of COD removed per day (kg-CODremoval/L); P_{coal} is the price of coal (RMB¥/m3); 0.25 is the conversion rate of COD and standard coal; 365 is the number of days in a year.

Figure 8-5 depicts the ECOD of VFL and AAO at different phases. The average temperatures of phases I, II and III were 34 ℃, 29 ℃ and 23 ℃, respectively, and the ECOD of VFL was significantly greater than that of AAO at the same influent volume and influent concentration, and the differences between VFL and AAO at different phases were 0.42 RMB¥/yr, 1.46 RMB¥/yr and 1.97 RMB¥/yr, which indicated that when the average temperatures were from 23 ℃ to 34 ℃, the removal efficiency of VFL for COD is significantly higher than the removal efficiency of AAO, so the environmental benefits generated by VFL at this phase are greater than those of AAO. The average temperatures of Phase IV and Phase V were 17 ℃ and 11 ℃, respectively, at which the ECOD of VFL was similar in scale to that of AAO, or even slightly lower than that of AAO, and the ECOD of both was significantly lower than that of Phase I to Phase III, which indicated that the temperature had an effect on the removal of COD by VFL and AAO. But the ECOD of AAO was slightly higher than that of VFL at this phase, and the hydraulic retention time of AAO was known to be greater than that of VFL, which indicated from another perspective that the HRT could be appropriately extended at lower temperatures to improve the removal efficiency of pollutants by VFL.

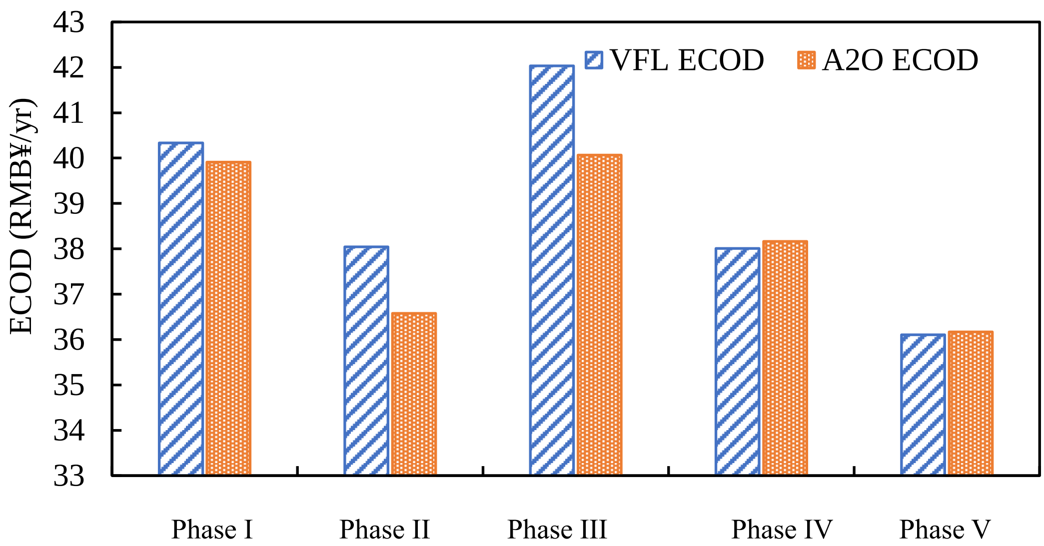

Figure 8-5. Comparison of ECOD of VFL and AAO.

The environmental benefits of ammonia nitrogen reduction are shown in Equation 1-3:

$$\mathrm{ENH_4^+ - N = QNH_4^+ - N \times 365 \times 2.14 \times P_{Urea}} \tag{1-3}$$

Where ENH_4^+-N is the environmental benefit of ammonia nitrogen (RMB¥/yr); QNH_4^+-N is the amount of COD removed per day, (kg-NH_4^+-Nremoval/L); P_{Urea} is the price of urea (RMB¥/m^3); 2.14 is the conversion rate of ammonia nitrogen and urea; 365 is the number of days in a year.

Figure 8-6 depicts the ENH_4^+-N of VFL and AAO at different phases, and it can be seen from Figure 1.6 that the difference between the ENH_4^+-N values of VFL and AAO at each phase is small, and the difference between the ENH_4^+-N of VFL and AAO at each phase is 1.68 RMB¥/yr, 0.91 RMB¥/yr, 1.75 RMB¥/yr, 2.48 RMB¥/yr, and 1.33 RMB¥/yr, respectively, which also indicates that the environmental benefits of VFL are higher than those of AAO in reducing ammonia nitrogen during the whole operation period. The difference between ENH_4^+-N of VFL and AAO was larger when the average temperature was 34 ℃ and 17 ℃, respectively, with the difference of 38.09 RMB¥/yr and 37.74 RMB¥/yr, which also indicated that the removal rate of ammonia nitrogen by VFL and AAO also decreased with the decrease of temperature.

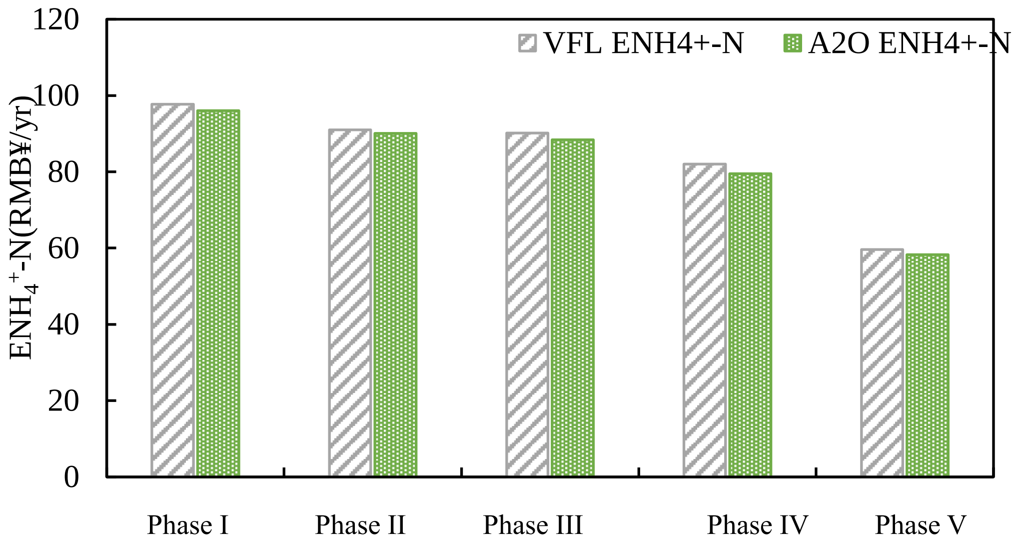

Figure 8-6. Comparison of E ammonia nitrogen of VFL and AAO.

Figure 8-7 depicts the Es of VFL and AAO at different phases. The trend of Es of VFL and AAO is similar to that of ENH_4^+-N, and the difference between them is also smaller: 2.11 RMB¥/yr, 2.37 RMB¥/yr, 3.72 RMB¥/yr, 2.33 RMB¥/yr, and 1.26 RMB¥/yr, respectively, which indicates that the difference in Es between VFL and AAO is smaller for the same influent volume and influent concentration, but VFL has a smaller footprint and lower hydraulic retention time than AAO, so the construction and operation costs of VFL are lower than those of AAO even though the Es generated are the same.

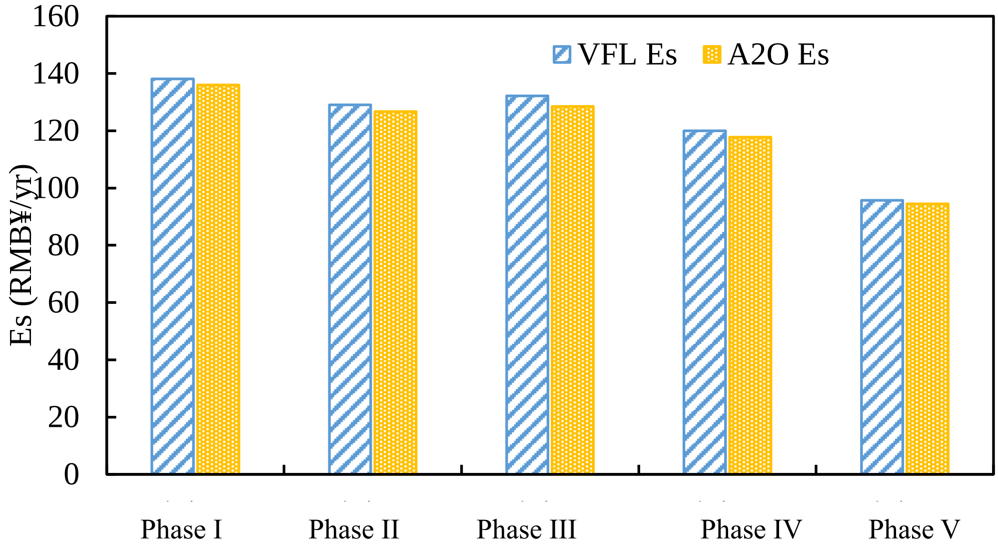

Figure 8-7. Comparison of Es of VFL and AAO.

The analysis of the ratio of ECOD to total ECOD for different phases is shown in Fig. 8-8. The ratio of ECOD to total ECOD for each phase of VFL is 20.70%, 19.60%, 21.60%, 19.50% and 18.60%; The ratios of ECOD to total ECOD for AAO were 20.90%, 19.10%, 21.00%, 20.00%, and 19.00%, respectively, which indicated that although there were some differences in the ratios of ECOD to total ECOD for different phases of VFL and AAO, under the same influent conditions, VFL was more efficient than AAO in removing COD and produced greater environmental benefits.

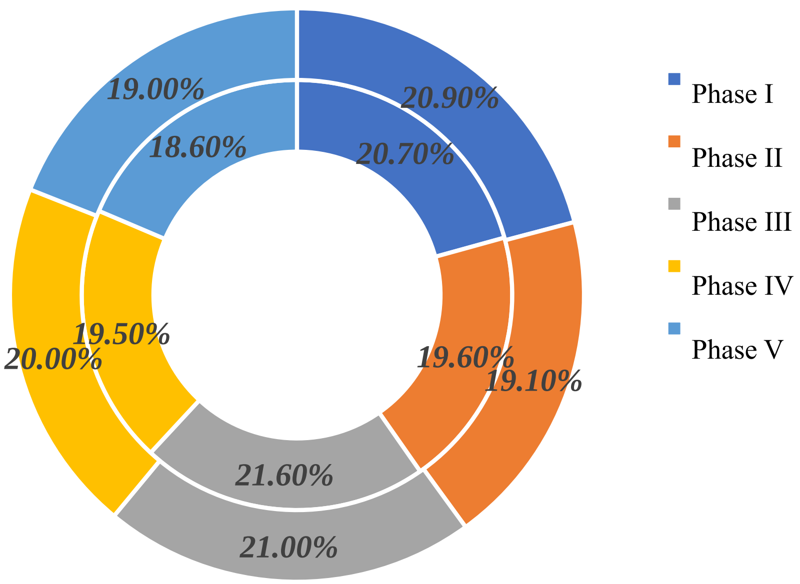

Figure 8-8. The Analysis of the Ratio of ECOD at Different Phases: VFL (Internal) and AAO (External).

The analysis of the ratio of ENH_4^+-N to total ENH_4^+-N at different phases is shown in Fig. 8-

9, and the ratios of ENH_4^+-N to total ENH_4^+-N for each phase of VFL were 23.20%, 21.60%, 21.40%, 19.50% and 14.20%, respectively. The ratios of ENH_4^+-N of AAO to total ENH_4^+-N were 23.30%, 21.80%, 21.40%, 19.30% and 14.10%, respectively.

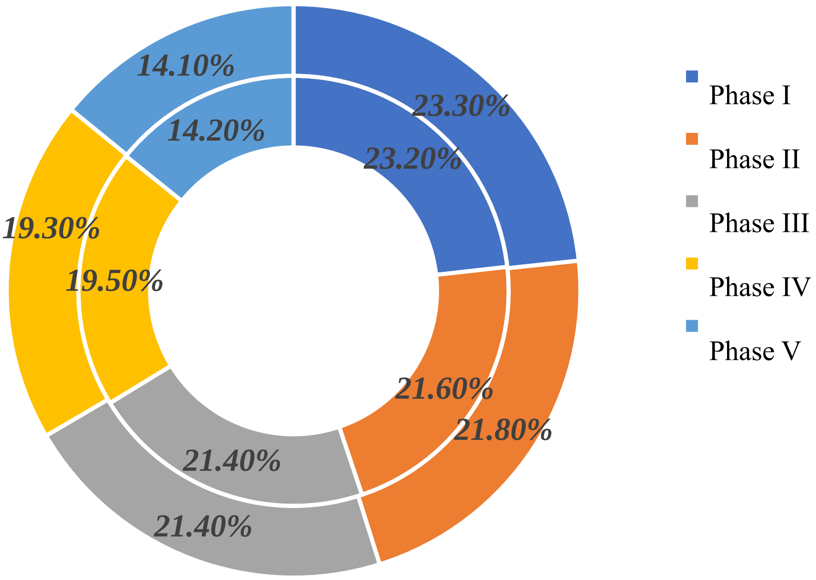

Figure 8-9. The Analysis of the Ratio of E Ammonia Nitrogen at Different Phases: VFL (Internal) and AAO (External).

The analysis of the ratio of Es to total Es for different phases is shown in Fig. 8-9, and the ratio of Es to total Es for each phase of VFL is 22.40%, 20.90%, 21.50%, 19.50% and 16.50%, respectively. The ratios of Es of AAO to total Es were 23.50%, 21.00%, 21.30%, 19.50% and 15.70%, respectively.

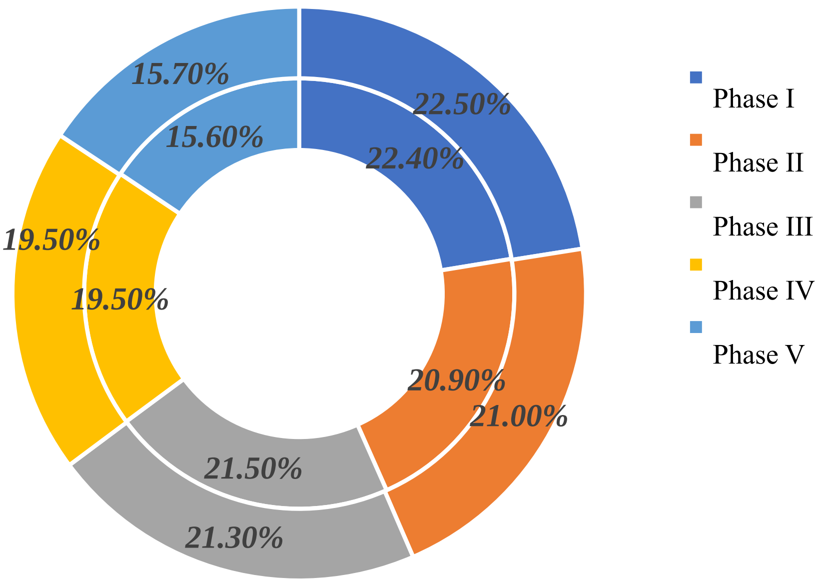

Figure 8-10. The Analysis of the Ratio of Es at Different Phases: VFL (Internal) and AAO (External).

By comparing the Es of VFL and AAO throughout the operation phases, the results show that the Es of VFL is greater than that of AAO in different phases, which also indicates that VFL is more effective in removing pollutants and brings more environmental benefits at the same influent concentration and influent volume. Simultaneously, since the hydraulic retention time and footprint of VFL is smaller than that of AAO, this also shows that VFL has higher environmental benefits and lower economic costs compared to AAO from the perspective of construction and operation costs. Therefore, VFL is more suitable as the primary choice for sewage treatment technology in green buildings.

8.4 Full life-cycle evaluation of VFL

The Life-Cycle (hereinafter referred to as LC) perspective originally originated in biology and narrowly refers to the process of birth, senility, illness and death of a particular organism, but nowadays it is also widely used in politics, economics, R&D and other fields, extending its broad concept: the whole period of a product from design to research, development, production, use to disposal, which is commonly known as the birth, senility, illness and death of a product, i.e., "from cradle to grave" [22, 23]. Different products have different LC. For example, the LC of electronic equipment may be 2 to 3 years, the LC of transport machines may be 4 to 10 years, and for housing construction, the LC may be 20 years or more. And the same product can vary in its composition under different perspectives. For example, from the producer's perspective, the product LC refers to the entire process starting from product design, through R&D, material acquisition, production and processing, acceptance and storage, to final sale to customers; however, from the customer's perspective, the product LC is the entire period of purchase, use, repair, and disposal. Typically the LC phases of the sewage treatment plant include the construction phase, the operating phase and the disposal phase [24].

Phase I: Construction. Sewage treatment is a large project, with large capital investment, large equipment size and large plant area, so the design planning and plant construction is very important. After obtaining the engineering construction permit, the sewage treatment project should start from the geographical survey, the selection of plant sites and technologies, the preparation of feasibility reports based on design and cost-benefit analysis, followed by bidding, raising funds, and finally carrying out plant construction, equipment acquisition, etc. Once the acceptance and trial run are passed, the project will officially enter the operating phase.

Phase II: Operating. A typical sewage treatment plant can operate for 15-30 years, during which the operating phase can be divided into operation work and maintenance work. The operation work is the most basic work to ensure that the sewage can be treated smoothly. The sewage treatment equipment at this phase is basically realizes automated treatment and automated monitoring, but it also requires regular monitoring by staff.

Phase III: Disposal. Once a sewage treatment project reaches the end of its LC, it is no longer continued, at which point the plant needs to be dismantled and restored to the environment. Equipment and fixed assets that can be recycled are disposed of and liquidated.

This study focuses on a brief analysis of the construction and operating phases of the VFL treatment technology for the selected treatment plant. And the average annual revenue was calculated based on the average annual project investment and operation fee obtained from the calculation.

8.4.1 Cost analysis of the VFL technology engineering construction

The construction costs for this study consider only the cost of the underlying physical facilities including the structures and equipment of the sewage collection and treatment system. Land purchase costs and initial working capital were not taken into account. The main factors considered when calculating the construction cost are shown by Equation 1-4.

$$Cap=Cap_s+Cap_t \qquad (1\text{-}4)$$

where Cap represents the cost of all equipment and structures for the project; Cap_s indicates the construction cost of the collection system; Cap_t is the sum of the input costs of the equipment and structures involved in the treatment system.

The collection system of this project mainly includes the pipeline construction for the process of gathering the wastewater production plant workshops to the sewage treatment plant, as well as the water collecting wells, sewage lifting pumps and other ancillary equipment for the storage of wastewater in the treatment plant. The whole LC cost of the collection system is 911,000 yuan according to statistics, of which the cost for pipeline construction is about 450,000 yuan, accounting for about 49.4% of the construction cost of the whole wastewater collection system. The cost analysis of the wastewater treatment system for this VFL sewage treatment technology analyzes the civil engineering and labor costs of the structures and the equipment procurement costs separately for subsequent calculations. The treatment units requiring LC cost analysis include the grille room, regulation tank, reaction tank, primary sedimentation tank, VFL combination pool, sedimentation tank, filter tank and sludge thickening tank, and other ancillary structures (blower room, dosing room, etc.). The treatment equipment involved mainly includes sewage lifting pumps, mixers, chemicals dosing systems, blowers and sludge thickeners. As can be seen from the results in Table 8-2, after a series of analyses, the total cost of the wastewater treatment system is about 53.788 million yuan, of which the investment in the construction of the VFL combination pool (about 34.6 million yuan) plus the aeration blower required for aerobic biological treatment (about 5 million yuan) dominates the overall project cost, which is about 73.6% of the total cost of the wastewater treatment system, which may be closely related to the volume of the combination pool and the degradation role it plays in the overall treatment process.

The engineering cost of the treatment technology is calculated from Equation (1-4) as 54.699 million yuan. As shown in Table 8-2, the treatment capacity of the sewage plant is about 5.1 million m^3/year, and the average engineering and construction investment per year is 1.727 million yuan, which is about 0.34 yuan/m^3 of wastewater.

Table 8-2. Cost of the whole LC.

Composition	Service life (year)	Remarks	Cost of the whole LC (1,000 yuan)
Collection system			**91.1**
Sewage lifting pump	10	3 sets	5.4
Water collecting well	50	Reinforced concrete structure	36.5
Pipelines	50		45
Others	10	--	4.2
Wastewater treatment system			**5378.8**
Grille	10	2 sets, B＝10 mm	4.3
Submersible mixer	10	2 sets, 3.0 kW	32.6
Sewage lifting pump	10	3 sets, 18.5 kW	5.4
Sewage lifting pump	10	1 set, 5.5 kW	1.8
Reaction mixer	10	3 sets, 3.0 kW	2.5
PAM dosing system	10	1 set	10.1
PAC dosing system	10	1 set	10.1
Mud scraper	10	1 set, 1.1 kW	3.5
Sludge pump	10	2 sets, Q＝125 m^3/h, 5.5 kW	10.3
Sewage lifting pump	10	9 sets, 7.5 kW	16.2
Mud discharge pump	10	3 sets, 2.2 kW	10.8
Machine mixer	10	1 set, 5.5 kW	1.2
Mud discharge pump	10	2 sets, 7.5 kW	7.2
Sewage pump	10	2 sets, 0.75 kW	8.6
Mud scraper	10	1 set, 1.5 kW	3.5
Backwash pump	10	2 sets	1.7
Blower	10	9 sets, 33 kW	500
Sludge thickener	10	1 set, 1.5 kW	26
Centrifugal dehydrator	10	2 sets, 45 kW	38
Fan	10	2 sets, 22 kW	3
Others (including automation instruments PLC, etc. and other ancillary equipment)	10		85
Grille well	50	15,000 m^3/d	13
Regulation tank	50	15,000 m^3/d	290
Reaction tank	50	15,000 m^3/d	25
Primary sedimentation tank	50	15,000 m^3/d	320
VFL combination pool	50	15,000 m^3/d	3460

Sedimentation tank	50	15,000 m^3/d	240
Filter tank	50	15,000 m^3/d	25
Sludge thickening tank	50	690m^3	74
Others	50		150
Total			**5469.9**

8.4.2 VFL technology operating cost analysis

Operating cost is the sum of annual expenses, including energy consumption, chemical consumption, sludge disposal, major maintenance and personnel costs. It can be calculated by Equation (2) that

$$Opc=Opc_e+Opc_c+Opc_s+Opc_m+Opc_p \qquad (1\text{-}5)$$

where Opc represents the operation cost; *Opc_e (energy consumption):* mainly the electrical energy consumption of each equipment; *Opc_c (chemicals consumption):* consumption of chemicals; *Opcs (sludge disposal cost):* including the cost of sludge treatment and transportation; *Opc_m (maintenance cost):* based on the set depreciable life; *Opc_p (staff cost):* staff benefits and salaries.

The main basic parameters during the operating process of the technology are shown in Table 8-3: Among them, energy consumption and chemicals consumptions are the main expense parts of the operating process. The electrical load of this project consists of a collection system and a sewage treatment system (including an odor treatment system), according to the load of the selected equipment, taking into account the lighting and ventilation power consumption, the total power consumption is about 4.01 million kWh/year, under the local pricing, the electricity price is 0.75 yuan/kWh. The prices of the main chemicals PAC and PAM are about 2,000 and 20,000 yuan/ton respectively, and the sludge disposal cost is 200 yuan/ton. In addition, the sewage treatment plant project has a service life of 50 years, according to the *Code for Seismic Design of Buildings* (GB50011-2010), the building site is designed for Class II, with a seismic intensity of 6 degrees, the selected equipment depreciable life is 10 years, the depreciation rate is calculated at 4.8%, and the total depreciation cost of buildings and equipment per year is about 2.63 million yuan (see Table 1.4). Combined with the actual consumption during technology operation, the annual operation cost is 19.3 million yuan, and the unit operation fee is 3.8 yuan/m^3 (see Table 8-3), i.e. it costs about 3.8 yuan/m^3 of wastewater treated during the operation.

Table 8-3. Basic Parameters of Project Operation.

Item	Unit	Value
Electricity price	yuan/kWh	0.75
Chemicals price: PAC	yuan/ton	2000
PAM		20000
Sludge disposal cost	yuan/ton	200
Depreciable life: Structures	Year	50
Equipment		10

Depreciation rate	%	4.8

8.4.3 VFL Engineering Net Profit Analysis

According to the operation cost and certain surplus capacity of this project, the revenue mainly comes from wastewater treatment and supplying reuse water to the enterprises in the park, and the charge for sewage treatment is set at 4.5 yuan/m^3, and the charge for reuse water supplied to the enterprises in the park is 0.3 yuan/m^3. The average annual treated water volume of this project is about 5.1 million cubic meters, and 50% of the treated water can be reused as industrial water, i.e., the reuse water volume is 2.55 million cubic meters, the annual profit is about 23.72 million yuan, of which the revenue from sewage treatment is 22.95 million yuan, accounting for 96.8% of the total annual revenue, while the revenue from reclaimed water reuse is 765,000 yuan, which is only 3.3% of the total revenue, indicating that the main source of revenue for the project is the fee levied for wastewater treatment within the plant.

The net revenue is calculated by the total revenue generated from wastewater treatment income, reuse water income, investment costs and operating costs [25, 26]. Based on the above analysis and calculation, the results are shown in Table 8-4. The annual investment cost is 1.727 million yuan, the total unit operation cost is 19.3 million yuan, and the annual profit is about 23.72 million yuan, so the annual net income is about 2.693 million yuan. The results show that a series of VFL-based wastewater treatment technologies are profitable as green buildings. More importantly, the profits will be even greater when the environmental benefits of treated and reused wastewater are taken into account.

Table 8-4. VFL Project Analysis of Treatment Plant Construction Cost, Operation Cost and Net Profit Analysis.

Item	Unit	Value
Influent water flow	10,000 m^3/year	510
Total investment	10,000 yuan	5469.9
Annual investment	10,000 yuan	172.7
Unit investment	yuan/m^3	0.34
Annual income	10,000 yuan	2372
Total annual operation costs	10,000 yuan	1930
Unit operation fee	Yuan	3.8
Depreciation cost	10,000 yuan	263
Annual net income	10,000 yuan	269.3

8.5 Evaluation of VFL as a sewage treatment technology in green buildings

VFL has advanced technology, mature process, stable effluent, high treatment efficiency, low operation cost, easy operation and management, less personnel required for operation, and stable effluent to meet the standard discharge. The VFL technology can effectively dispose of pollutants in the sewage treatment process, effectively avoiding secondary pollution.

8.5.1 Technology analysis of VFL as reclaimed water reuse in green buildings

The correct choice and reasonable combination of reclaimed water treatment technology is of vital importance for the normal operation and treatment effect of reclaimed water system. Therefore, the selection of reclaimed water treatment technology should pay attention to the following points. 1) The quality and quantity of raw water, and the effluent quality and quantity of reclaimed water. 2) The requirements of use and the actual situation of the project. 3) Technical and economic comparison under the premise of meeting the specification requirements. Therefore, it is advisable to adopt mature technologies with the high technical integration, stable and feasible operation, and good cost performance in green buildings.

8.5.1.1 Process analysis of VFL technology

The specific process diagram of VFL as the main treatment technology of sewage treatment plant is shown in Fig. 8-11.

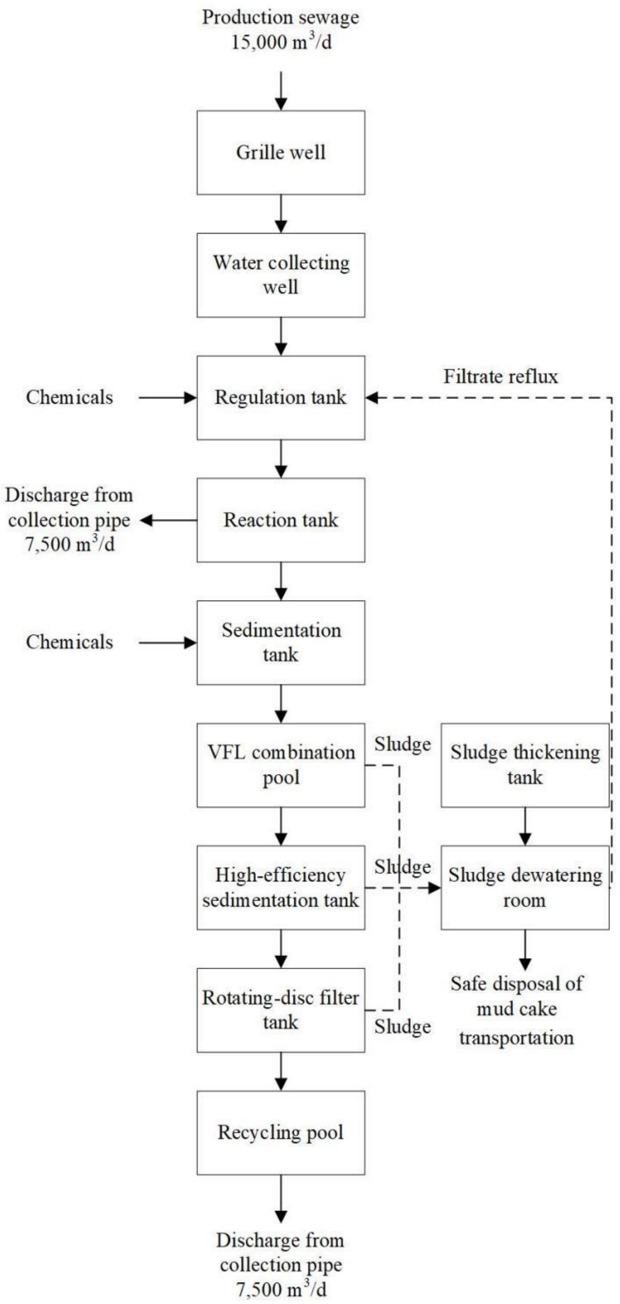

Figure 8-11. Process Diagram of VFL.

Since the existing sewage plant was put into service, it has been operating normally, with an average daily treatment capacity of about 15,000 m^3/d and a peak treatment capacity of 20,000 m^3/d, and an average daily reuse water capacity of 7,500 m^3/d after treatment.

8.5.1.2 Feasibility study content for technology selection

The feasibility study for this project was prepared on the basis of a full investigation and study.

1. Scope of study. The project design scale, sewage quality, project site selection, and sewage/sludge treatment technology have been compared and demonstrated in terms of technical

reliability, economic rationality, and implementation possibility of multiple options. On this basis, a scientific and reasonable, technologically advanced, excellent treatment effect, stable and reliable operation, small footprint, cost saving and low operation cost scheme is proposed to make the project play the best social, environmental and economic benefits.

2. Main contents. The project scale, influent and effluent quality, sewage treatment technology, reuse water treatment technology, sludge treatment and disposal technology, project investment, technical and economic indicators, etc. are demonstrated.

3. Preparation principle.

(1) Strictly implement the relevant national and local environmental protection regulations and economic and technical policies, combine with the actual local situation to prepare this feasibility study report according to local conditions.

(2) With the comprehensive consideration of the overall planning of the development of the children's clothing industry park, the existing sewage treatment plant in the industrial park is reasonably connected. The plan layout is reasonable and compact, so that the project and the gathering area plan are consistent.

(3) Select the sewage treatment technology with advanced technology, mature process, stable effluent, high treatment efficiency, low operation cost, convenient operation and management, and less personnel required for operation, to ensure the effluent is stable and meets the standard discharge.

(4) Choose technologically advanced, energy-saving and reliable technologies and equipment at home and abroad, and reasonably support advanced and reliable automatic control systems to ensure the safe operation of the sewage treatment plant and improve the operation and management.

(5) Treat and dispose the sludge produced in the sewage treatment process properly to avoid secondary pollution.

8.5.2 Application of VFL effluent water as green building reuse water

To improve the utilization of water resources, the effluent water from the VFL system can be reused in the city's production, and the daily reuse amount is 7500 m^3/d, and the design effluent quality based on the sewage treatment plant is shown in Table 8-5.

Table 8-5. Design Effluent Quality.

Name	pH	CODcr (mg/L)	BOD_5 (mg/L)	SS (mg/L)	NH_3-N (mg/L)	TP (mg/L)	TN (mg/L)	Chromaticity (times)
Discharge water quality	6~9	≤200	≤50	≤100	≤20	≤1.5	≤30	≤80

A summary of the reuse water quality is shown in Table 8-6.

Table 8-6. Design reuse water quality.

Name	Reference water quality (GB/T19923-2005) Washing water quality	Reuse water quality
pH	6.5~9.0	6.5~9.0
CODcr（mg/L）	/	≤100
BOD_5（mg/L）	≤30	≤30
Chromaticity (dilution ratio)	≤30	≤30
SS (mg/L)	≤30	≤10

It can be seen that the effluent quality of this system meets the reuse standard, and the water reused by the city production can now be used for greening and irrigation, road flushing and garage flushing, etc. The effluent water from the sewage treatment technology in green buildings can also be reused in greening and irrigation.

In the green building community, the combination of sprinkling irrigation and watering is used to green irrigation according to the field conditions, with pipelines to deliver pressurized water to the irrigation lot and dispersing it into tiny droplets through the sprinkler head to irrigate the greenery plants evenly. The green area with small area and less accessible to lay pipelines is watered by manual watering, see Fig. 8-12.

Figure 8-12. Greening and Irrigation Flow Chart.

There are several advantages of sprinkling irrigation [27]. (1) It is effective in saving water, with water utilization rate up to 80%. In general, 1 m^3 of sprinkling irrigation is twice the effect of the same amount of the surface irrigation. (2) The green plants grow well, the sprinkling irrigation is evenly applied without soil caking, which is conducive to the growth of green plants, and improves the green space microclimate and living ecological environment. (3) It greatly reduces the workload of the property department in watering pipe system construction and management and maintenance. (4) It avoids secondary salinization of soil caused by excessive irrigation and undesirable phenomena such as rotten roots of green plants.

8.5.3 Evaluation of VFL as a sewage treatment technology in green buildings

8.5.3.1 Technology selection evaluation

This technology is built in comprehensive environmental management supporting park in Huzhou, China. The treatment technology adopts "coarse grille well + water collecting well + fine grille well + regulation tank + floatation tank", with the VFL biochemical treatment technology and "immersion ultrafiltration" reuse water treatment technology.

(1) The VFL has advanced technology that is safe and applicable, and the effluent water from the reactor can meet the water quality standards for reuse.

(2) VFL is economically reasonable, with low investment, operation fee and floor space while ensuring the effluent quality.

(3) VFL has high pollutant removal efficiency and high environmental benefits.

It can simultaneously accomplish the functions of organic matter removal, nitrification denitrification, and phosphorus removal by excessive uptake. The premise of denitrification is that NH3-N should be completely nitrated, both aerobic and deoxygenation tanks are operating properly, and it is possible to combine the two for phosphorus removal. The advantages of this method are as follows.

1. Excellent and stable treatment. It can decompose the particulate matter in water into dissolved organic matter, convert the hard-to-degrade macromolecules into small molecules, and remove most of the chromaticity in water.

2. Strong anti-shock load. The internal reflux capacity is 2~3 times of the influent capacity, with a large dilution homogenization capacity and strong anti-shock load.

3. Low sludge volume. Due to the long age of biological sludge and low sludge load, the synthetic sludge can be stabilized in the aerobic tank with low sludge production.

4. The technology is mature and reliable, with a wide range of use. This technology achieves perfect treatment results in most areas in the north and south of China.

5. It is capable of removing organic matter, ammonia nitrogen and phosphorus simultaneously. The organic cooperation of three different environmental conditions of anaerobic, anoxic and aerobic and different kinds of microbial flora can remove organic matter, nitrogen and phosphorus at the same time.

6. Low operation fees and easy maintenance. Hydrolytic acidification promotes the biochemical properties of the wastewater and reduces the aerobic residence time and operation fees. And under anaerobic conditions, filamentous bacteria tend to be less prolific, with SVI generally less than 100, and no sludge expansion occurs, allowing for easy management.

8.5.3.2 Location evaluation

This project is located in the eastern part of Huzhou City, which belongs to the plain water network area, and the surrounding terrain is generally flat, without hills and mountains. The region is located at mid-latitude, with long winters and summers and short springs and autumns. The local climate is hot in summer, cold and dry in winter, variable in spring and autumn, cloudy and rainy in spring, wet and then dry in autumn. Details of temperature, precipitation and sunshine are shown in Table 8-7.

Table 8-7. Statistics of Meteorological Observations in Huzhou.

S/N	Meteorological factors	Statistical value
1	Annual average temperature	15.2 ℃
2	Average temperature of the hottest month	27.2 ℃
3	Average temperature of the coldest month	3.3 ℃
4	Annual average precipitation	1248 mm
5	Annual average precipitation days	144 d
6	Annual average sunshine hours	2074 h
7	Annual frost-free period	224~246 d

8.5.3.3 Material selection evaluation

All the buildings of this project are as follows. Sludge yard (18.0×8.0×1F), fan room (12.0×20.0×1F), distribution room (12.0×15.0×1F), dosing room (12.0×5.0×1F), duty room (10.0×5.0×1F), water supply pump room (16.0×6.0×1F) and online monitoring room (6.0× 4.0×1F).

1. Structural type: All buildings in this project are made of brick.

2. Building materials. The beams and columns in the buildings of this project are all made of C25 concrete, with HRB400 rebar. Main materials such as steel, cement, welding rod and bricks without factory certificate and test certificate shall not be used. The walls in the building are required for drip lines, featuring straight, neat and polished surfaces.

3. Fire-fighting measures. The selection of interior decoration materials for this project building must meet the requirements of fire prevention, and according to the actual situation, A1 grade non-combustible materials or B1 grade non-combustible materials shall be selected. The interior of each building should be equipped with fire extinguishers under the current national standard *Code for Design of Extinguisher Distribution in Buildings* (GB50140-2010).

4. Light and ventilation measures. According to the requirements of light and ventilation, each building of this project is equipped with windows, and the window size types are 1500×1500 mm.

8.5.3.4 Engineering construction evaluation

This technology follows the following principles in the construction process.

1. It is designed to be "safe and applicable, economical and beautiful, harmonious and unified", focusing on the practicality and economy of the building, balancing aesthetics, and unifying with

the overall architectural style of the plant area.

2. With the framework and other structural forms, according to local conditions, priority was given to the use of local materials and industrial waste to reduce capital construction funds.

3. Optimize the choice of plant area, span, column spacing, height, and properly deal with technical issues such as waterproofing and anti-corrosion of buildings and structures.

4. According to the Standard for Architectural Drawings GB/T50105-2010 and GB/T50105-2010, as well as the Unified Standard for Building Drawing.

5. Comply with the national and provincial design technical regulations and specifications related to the project.

6. The unit of measurement is millimeter (mm), except for the elevation, which is in meters (m).

7. The physical, chemical and mechanical properties of the selected construction, decoration materials and building accessories should meet the design requirements, some of which should also ensure the quality of appearance.

8.5.3.5 Construction cost evaluation

After the completion of this project, the total treatment scale of children's clothing industrial park sewage treatment project is 15,000 m^3/d, and the total treatment scale of reuse water is 7,500 m^3/d. This financial evaluation is calculated on the premise that the total sewage treatment scale of the industrial park is 15,000 m^3/d after the completion of the project.

(1) Cost estimation basis

1. Original value of fixed assets. Based on the sum of the first part of the project cost, joint commissioning cost, reserve cost, and loan interest during the construction period in the fixed asset investment.

2. Depreciation costs of fixed assets. The original value of fixed assets depreciates at a rate of 4.8%.

3. Amortization of the intangible and deferred assets. The cost of the second portion of the investment in fixed assets is amortized over 10 years in accordance with industry regulations.

4. Maintenance costs. The maintenance costs of this project include daily maintenance and membrane replacement. The daily maintenance costs include routine equipment maintenance, building repair and maintenance, accessories replacement, etc. The daily maintenance costs are calculated as 10% of the total equipment investment, and the membrane replacement costs are calculated according to the 3-year service life of the ultrafiltration membrane.

5. Management and other costs. Calculated as 10% of the sum of the above-mentioned costs.

6. Basic data. The basic data needed for cost estimation are shown in Table 8-8.

7. Energy consumption. The energy consumption during the production and operation of this project is mainly water, electricity and chemical consumption, and the annual energy consumption of the whole plant after commissioning is shown in Table 8-9.

Table 8-8. Cost Basis Data Table.

S/N	Item	Value
1	Electricity price	0.75 yuan/kwh
2	Water price	2.5 yuan/ton
3	Chemicals price	
(1)	PAC (10% concentration liquid)	400 yuan/ton
(2)	PAM (solid)	28,000 yuan/ton
(3)	Caustic soda liquid (30% concentration liquid)	1,000 yuan/ton
(4)	Sodium hypochlorite (10% concentration liquid)	500 yuan/ton
(5)	Citric acid (50% concentration liquid)	5,300 yuan/ton
4	Sludge disposal cost	350 yuan/ton
5	Ultrafiltration membrane replacement cost	2.6 million yuan/year
6	Average annual salary and benefits of employees	50,000 yuan/year
7	Depreciable life	50 years for building structures and 10 years for equipment
8	Residual rate	4%
9	Amortization of the intangible and deferred assets	10% (intangible)
10	Income tax rate	25%
11	Calculation period of the project	20 years (including 1 year construction period)
12	Designer quota	23 people (including 16 existing employees and 7 new employees)
13	Design sewage treatment capacity	$25000m^3/d$
14	Design reuse treatment capacity	$12500m^3/d$

Table 8-9. Annual Energy Consumption of the Whole Plant after Commissioning.

S/N	Name	Applications and specifications	Dosage	Source of supply
1	Water	Production and living	8000 t /a	Tap water
2	Electricity	/	6,253,000 kWh/year	Power supply system
3	Chemicals	PAC (10% concentration liquid)	12375.0t/a	Manufacturer
4	Chemicals	PAM (solid)	24.75t/a	Manufacturer
5	Chemicals	Caustic soda liquid (30% concentration liquid)	5000.0 t/a	Manufacturer
6	Chemicals	Citric acid (50% concentration liquid)	19.8 t/a	Manufacturer
7	Chemicals	Sodium hypochlorite (10% concentration liquid)	26.4t/a	Manufacturer

(2) Cost estimation

The total cost estimate of this project is detailed in Annexes 4. The main indicators are as follows.

1. Total cost of sewage treatment (average): 3,809,300,000 yuan/year

2. Sewage treatment unit water production cost: 4.84 yuan/m^3

3. Sewage treatment unit water production operating cost: 3.81 yuan/m^3

Note: The sewage charges levied by relevant authorities are not included in the above costs.

In summary, VFL is a quality choice as a sewage treatment technology in green buildings. The five aspects of VFL technology are evaluated through technology selection evaluation, location evaluation, material selection evaluation, engineering construction evaluation and construction cost evaluation, which helps to understand the operation and construction of VFL technology in a comprehensive manner. The coverage of the evaluation is shown in Fig. 8-13.

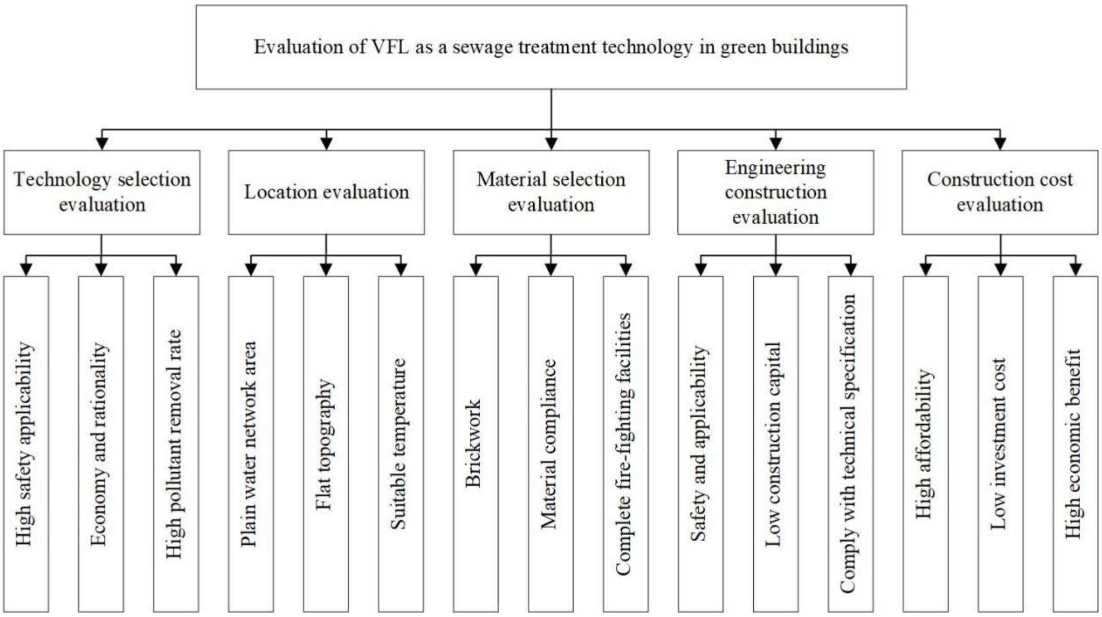

Figure 8-13. Evaluation of VFL as a Sewage Treatment Technology in Green Buildings.

8.6 Summary

Green building can effectively integrate resources and environmental issues, so as to solve the problem of harmonious development of human and environment to the greatest extent. At present, green buildings mainly recycle water resources through reclaimed water reuse and rainwater reuse. The VFL technology and the AAO technology have high pollutant removal rates and high environmental benefits in the operation of sand washing wastewater treatment, so VFL is a good choice for green building sewage treatment technologies. The engineering construction and operation phases of the VFL treatment technology of the selected treatment plant were analyzed based on the whole LC theory. The results show that the engineering cost of the treatment technology is 54,699,000 yuan, and the average annual engineering and construction investment is 1,727,000 yuan, which is about 0.34 yuan/m^3 of wastewater. The calculation of operation costs takes into account energy consumption, chemical consumption, sludge disposal, major maintenance and personnel costs, combined with the actual consumption during technology operation, the annual operation fee is 19.3 million yuan, and the unit operation fee is 3.8 yuan/m^3. The revenue of this project comes mainly from wastewater treatment and supplying reuse water to enterprises in the park, with a total annual profit of about 23.72 million yuan. In addition, the average annual revenue is calculated to be 2,693,000 yuan based on the combined annual project investment, annual operation expenses and total profit. The above results show that a series of VFL-based wastewater treatment technologies are profitable as green buildings. More importantly, the profits will be even greater when the environmental benefits of treated and reused wastewater are additionally taken into account. VFL can be used as a treatment technology for green building sewage reuse. The effluent quality of VFL meets the reuse standard and is mainly used for greening and irrigation. The VFL technology is evaluated in five aspects: technology selection evaluation, location evaluation, material selection evaluation, engineering construction evaluation and construction cost evaluation, and is hereby recommended for use as the sewage treatment technology in green buildings.

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Chapter 9

CONCLUSION

CONCLUSION

CHAPTER 9: CONCLUSION

9.1 Main conclusion

In this study, the performance of the VFL device for landfill leachate and sand washing wastewater treatment was investigated, the effect of the VFL device and AAO process on sand washing wastewater treatment was compared, the microbiological mechanism of the VFL device for landfill leachate treatment was analyzed, and the VFL device and AAO process were compared. Microbial community differences in sand washing wastewater treatment by AAO process. The main conclusions are as follows:

1) The VFL treatment process for high-concentration landfill leachate is economical and reasonable and has high treatment efficiency. The VFL device can effectively remove the high COD concentration, and the removal rate can reach up to 86.5%. The effluent COD was stable in the whole stage, and the concentration fluctuation of the influent did not affect the effluent COD concentration. The average concentration of NH_4^+ in the effluent is 15.5 mg/L, which meets the requirements of China's national secondary emission standard, where the NH_4^+ concentration is lower than 25 mg/L, and the NH_4^+ removal rate is as high as 99.3%. The degradation effect of total phosphorus is obvious. The total phosphorus concentration in each stage was relatively stable. The conductivity of the influent water is significantly higher than in other stages. The VFL device degrades volatile fatty acids.

2) During the VFL treatment of landfill leachate, the MLSS fluctuated greatly in August and September. Compared with the anaerobic stage, the MLSS of the anoxic stage increased by 9%; that is, the activity of microorganisms in the activated sludge was improved. MLVSS in anoxic section (8899.7 mg/L)>anaerobic section MLVSS (8458.8 mg/L)>aerobic section MLVSS (8205.6 mg/L). The MLVSS of the anoxic section is the largest and combined with the above MLSS; it is speculated that the environmental conditions of the anoxic section provide a more suitable attachment site for the microorganisms, accelerate the metabolism of the microorganisms, and thus promote the increase of the MLVSS of the sludge mixture, and the growth rate is the fastest. MLVSS/MLSS maintained at 0.72-0.75, indicating that the activated sludge in the system maintained a high activity. The activated sludge in the anaerobic, anoxic, and aerobic stages all have irregular shapes, irregular edges, and brown color, and the color depth depends on the density of the sludge. Moreover, the activated sludge is flocculent, the internal density of the sludge is uneven, the center density is large, the edges are sparse, and there are still many flocs around the sludge that gather towards the center of the sludge. The sludge in the anoxic stage was denser, while the sludge in the aerobic stage was relatively sparse.

3) Metagenome sequencing technology was used to analyze the microbial community structure of the sludge samples in the anaerobic, anoxic, and aerobic sections of the long-running VFL unit to treat landfill leachate. In general, as the seasonal temperature decreases, it is not conducive to the

reproduction of microorganisms involved in the degradation of landfill leachate, which leads to a relative reduction in the microbial diversity in the VFL device. Proteobacteria, Actinobacteria, unclassified_Bacteria, Deinococcus-Thermus, Chloroflexi, Ignavibacteriae, Planctomycete at the phylum level Bacteroidetes were the dominant flora. At the genus level, *Thauera*, *Ignavibacterium*, *Nitrosomonas*, *Truepera*, *Pseudofulvimonas*, and *Lewinella* were the dominant communities. The bacterial sequences in the VFL device can predict 47 gene functions. In addition to the strong essential functions of maintaining cell life activities, nitrification and denitrification, photoautotrophy and photoabnormality, and carbohydrate transport and metabolism functions. The aspect potential is also relatively high in the proportion of functions. However, with the decrease in seasonal temperature, the microbial function decreased and denitrification decreased, but methyl nutrition was abnormally abundant.

4) After VFL treatment, BOD_5 in sand washing wastewater was effectively degraded, and the average degradation rate of BOD_5 was as high as 86.3%. BOD_5 tends to be stable in each sampling section of VFL, and the change range is not extensive. The NH4+ concentration at each stage was relatively stable during the sampling period. The average removal rate of total nitrogen in the anaerobic stage was 44.3%, the average removal rate of total nitrogen in the anoxic stage was 66.8%, the average removal rate of total nitrogen in the aerobic stage was 77.8%, and the average removal rate of total nitrogen in the effluent was 80%. Overall, the total nitrogen removal rate was better during the long-term stable operation. The total phosphorus degradation rate in the effluent reached 66.7%. The pH in the VFL device did not change much and was relatively stable.

5) The BOD_5 of sand washing wastewater has a small fluctuation range in the effluent stage, aerobic stage, and anoxic stage of the AAO process; however, in the influent and anaerobic stages, the BOD_5 fluctuation range is large. When the AAO process treats sand washing wastewater for a long time, the ratio of BOD_5 to COD in the inlet section is always greater than 0.3, which indicates that biological methods can effectively treat the sand washing wastewater. Compared with the concentration of NH_4^+-N in the influent, the NH_4^+-N in the effluent was effectively degraded, the degradation rate reached 88%, and the degradation effect was better. NO_3^--N effluent concentration is higher than influent concentration. NO_3^--N changes very smoothly in the influent section, anaerobic section and anoxic section. On the contrary, NO_3^--N fluctuates significantly in the aerobic section and the effluent section. The degradation rate of phosphorus in the effluent section reached 66.7%. Long-term monitoring of pH found that the average pH of the inlet section was 6.6, the average pH of the anaerobic section was 6.2, the average pH of the anoxic section was 6.7, the average pH of the aerobic section was 6.5, and the average pH of the effluent section was 6.5. The pH average is 6.6.

6) The Simpson index of each stage of the VFL device is smaller than that of the AAO process. In addition, the Coverage of all samples is greater than 99%, indicating that the sequencing results can represent the real situation of the samples. Proteobacteria was the main dominant bacterial phylum, which was the most in the aerobic section of the VFL device, reaching 77.76%. The

microbial communities of the VFL plant and the AAO process were different at the family level. Sphingomonadaceae were detected in the highest number in the AAO process. Anaerolineaceae (phylum Chloroflexi) predominate in VFL installations. *Novosphingobium* has a major advantage in the AAO process. *unclassified_Rhodocyclaceae* had a larger proportion in VFL. In addition, anammox functional genes, genes involved in nitrification, denitrification and nitrogen fixation were detected.

7) At present, green buildings mainly recycle water resources through reclaimed water reuse and rainwater reuse. The VFL technology and the AAO technology have high pollutant removal rates and high environmental benefits in the operation of sand washing wastewater treatment. The engineering construction and operation phases of the VFL treatment technology of the selected treatment plant were analyzed based on the whole LC theory. The results show that the engineering cost of the treatment technology is 54,699,000 yuan, and the average annual engineering and construction investment is 1,727,000 yuan, which is about 0.34 yuan/m^3 of wastewater. The calculation of operation costs takes into account energy consumption, chemical consumption, sludge disposal, major maintenance and personnel costs, combined with the actual consumption during technology operation, the annual operation fee is 19.3 million yuan, and the unit operation fee is 3.8 yuan/m^3. The revenue of this project comes mainly from wastewater treatment and supplying reuse water to enterprises in the park, with a total annual profit of about 23.72 million yuan. In addition, the average annual revenue is calculated to be 2,693,000 yuan based on the combined annual project investment, annual operation expenses and total profit. More importantly, the profits will be even greater when the environmental benefits of treated and reused wastewater are additionally taken into account. VFL can be used as a treatment technology for green building sewage reuse. The effluent quality of VFL meets the reuse standard and is mainly used for greening and irrigation. The VFL technology is evaluated in five aspects: technology selection evaluation, location evaluation, material selection evaluation, engineering construction evaluation and construction cost evaluation, and is hereby recommended for use as the sewage treatment technology in green buildings.

9.2 Analysis of the innovation point of this paper

First, for high-concentration refractory landfill leachate, a VFL treatment method was proposed, and its long-term operation feasibility and stability were investigated;

Second, the changes in the microbial community structure in the VFL device were explored to reveal the biological removal mechanism of pollutants.

Third, the performance of the VFL device and the existing AAO process in treating sand washing wastewater were compared, and the mechanism was revealed at the molecular biology level.

9.3 Deficiencies and prospects

This study shows that the VFL device has a specific treatment effect on sewage. However, few discussions on the operating conditions, such as the proportion of influent water and dissolved oxygen. In the future, the working conditions and operating parameters can be optimized further to improve the pollutant removal effect of the device. In addition, life cycle assessment can explore further the operation cycle and social benefits of VFL devices. This study found that the VFL device operates in northern China and is easily affected by the seasonal temperature. It is possible to explore further the size of the impact on various water bodies and the improvement measures.